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Pyrrolobenzodiazepines as Sequence Selective DNA Binding Agents

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1. Introduction

The heritability of cancers is usually affected by complex interactions between carcinogens and the host's genome. New aspects of the genetics of cancer pathogenesis, such as DNA methylation, and micro RNAs are increasingly recognized as important events. Chemotherapeutic drugs interfere with cell division in different ways, e.g. with the duplication of DNA or the separation of newly formed chromosomes. Most forms of chemotherapy target rapidly dividing cells and are not specific for cancer cells, although some degree of specificity may come from the inability of many cancer cells to repair DNA damage. Hence, chemotherapy has the potential to harm healthy tissues, especially those tissues that have a high replacement rate (e.g. intestinal lining). These cells usually repair themselves after chemotherapy.^[1] DNA has long been considered a favored target for cancer chemotherapeutic agents. Indeed many of the most effective clinical agents, such as alkylating and interactive agents, are DNA interactive. Achieving the desired sequence specificity with DNA-interactive agents is considered to be one of the most formidable hurdles in the development of new agents to achieve therapeutic invention.^[2]

Certain structural features of the alpha helix are particularly important when considering drug-DNA interaction. Double helical DNA is not a uniform structure, there are places where the strands are further apart, and where they are closer together, these are known as major and minor grooves. In B-DNA, the major groove is wider (12 versus 6 Å) and deeper (8.5 versus 7.5 Å) than the minor groove, making it more accessible to interacting molecules. The base pair arrangement for each groove is very specific, each containing certain hydrogen bond donors and acceptors. In addition, the major groove will also contain the methyl group of thymine and drug molecules or proteins bind at these sites. More importantly the difference between the donor and acceptor groups in each groove, this makes it possible for drug molecules to selectively distinguish between the different bases as well as sequences of bases.^[3,4]

2. DNA groove binders

Drugs that bind to DNA may occur on the major groove face, minor groove face or a combination. The grooves are excellent sites for sequence specific recognition since there are

many potential hydrogen bond donor and acceptor atoms unique to each base pair combination along the base edges. The greater width associated with the B-DNA major groove makes the major groove a somewhat more preferable binding groove. Groove binding can be via the major or minor groove and covalently or non-covalently. Most DNA interactive proteins bind in the major groove, while small molecules of less than 1000 Da, including many antibiotics, bind in the minor groove.

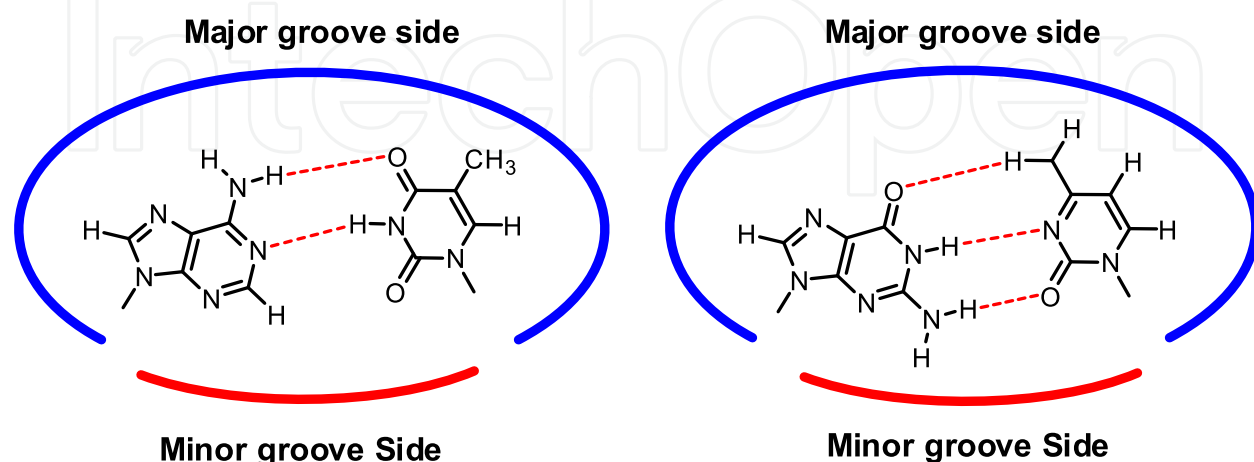


Fig. 1. Hydrogen bonding between adenosine/thymine (left) and guanosine/cytosine base pairs of DNA.

The minor groove represents a vulnerable site of attack in that it is normally unoccupied, and this is presumably the reason for the evolution of antibiotics that attack the DNA of competing organisms. Thus, although at first sight minor groove binders are less attractive as probes in that they target the less information rich minor groove nevertheless, they may prove to have several advantages compared with major groove ligands. The development of sequence-specific probes based on naturally occurring DNA groove-binding agents is, therefore, an alternative and complementary approach to the antisense oligonucleotide strategy. The main motive for synthesizing a large number of analogues and conjugates of naturally occurring minor groove-binding agents, is to generate new lead compounds with potential anticancer properties and specific DNA sequence recognition.^[5]

2.1 Covalent binding in the minor groove of DNA

Drugs which bind covalently to DNA are used to either add substituents onto base residues, or to form cross-links between different sections of DNA. The first mechanism results in a base-pairing mismatch during DNA replication, and the DNA is ultimately fragmented by the enzymes which try to repair it. The second mechanism binds together the two strands of DNA helix, preventing separation during the replication process. Electrophilic functional groups such as epoxides, aziridines, carbinolamines, imines and cyclopropanes are found in a variety of synthetic and natural products capable of covalent interaction with DNA; examples include mitomycin, saframycins and pyrrolobenzodiazepines (anthramycin).^[6] Non-covalent binding compounds are typically isohelical with B-DNA and fits snugly within the minor groove, held in a position by a combination of hydrogen bonds, van der Waal forces and electrostatic interactions; examples include distamycin,^[7] netropsin,^[8] lexitropsins and bis-benzimidazole (Hoechst 33258).^[9]

2.2 Pyrrolo[2,1-c][1,4]benzodiazepines (PBDs) as DNA binding agents

Binding of low molecular weight ligands to DNA causes a wide variety of potential biological responses. In this context pyrrolo[2,1-c][1,4]benzodiazepines (PBDs), a group of potent naturally occurring antitumour antibiotics isolated from various *Streptomyces* species, are one of the promising type of lead compounds. They differ in the number, type and position of substituent in both their aromatic A-ring and pyrrolidine C-ring, and in the degree of saturation of the C-ring which can be either fully saturated or unsaturated at either the C2-C3 (endocyclic) or C2 (exocyclic) positions. There is either an imine or carbinolamine methylether moiety at the N10-C11. Position, which is an electrophilic centre responsible for alkylating DNA. To date, thirteen structures isolated, include compounds like anthramycin,^[6] mazethramycin,^[10] porothramycin,^[11] prothracarcin,^[12] sibanomycin,^[13] tomaymycin,^[14] sibiromycin,^[15] chicamycin A,^[16] neothramycin A, B^[17] and DC-81^[18] (Figure 2).

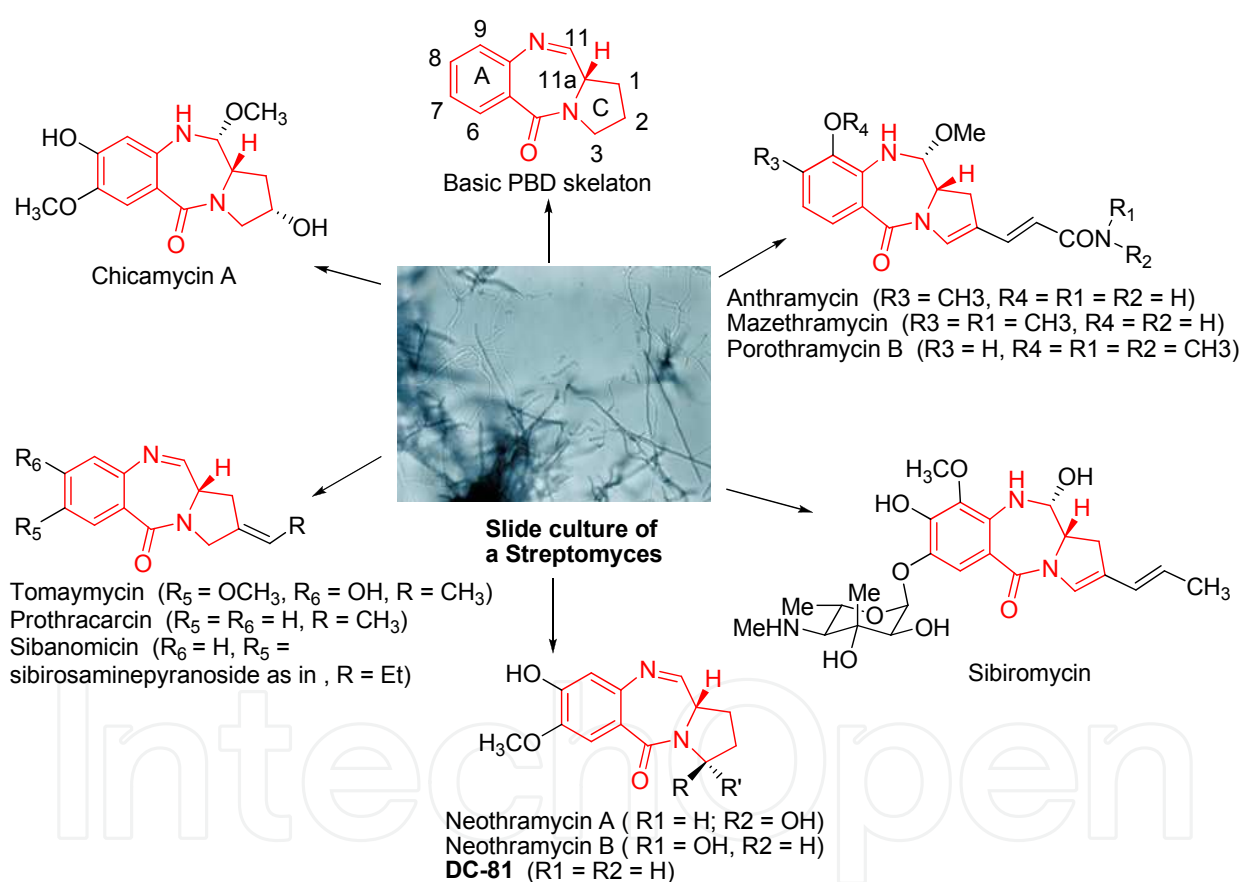


Fig. 2. Naturally occurring PBDs.

The PBD interactions with DNA are unique since they bind within the minor groove of DNA forming a covalent amination bond between the C11-position of the central B-ring and the N2 amino group of a guanine base.^[19] The cytotoxic and antitumour activity of PBDs are attributed to their ability to form covalent DNA adducts. Molecular modeling, solution NMR, fluorimetry and DNA foot printing experiments have shown that these molecules have a preferred selectivity for Pu-G-Pu sequences^[14,20] that are oriented with their A-rings pointed either towards the 3' or 5' end of the covalently bonded DNA strand (as in case of

anthramycin and tomaymycin). The PBDs have been shown to interfere with the action of endonuclease enzymes on DNA^[21] and to block transcription by inhibiting DNA polymerase in a sequence specific manner,^[22] processes which may be relevant for the biological activity.

The known PBD natural products have a (*S*) configuration at the C11a-position, which provides them with a right-handed twist when viewed from the C-ring towards the A-ring. This has given the appropriate three-dimensional shape for isohelicity with the minor groove of DNA, leading to a snug fit at the binding site. Recemization at C11a can significantly reduce both DNA binding affinity and *in vitro* cytotoxicity. A synthetic PBD with the (*R*)-configuration at C11a was shown to be devoid of both DNA binding affinity and *in vitro* cytotoxicity.^[23] The N10-C11 imine moiety may exist in the hydrated form depending upon precise structure of the compound and the method of isolation or synthetic workup. Imines and methyl ether forms are interconvertable by dissolution of imine in methanol or by several cycles of refluxing the methyl ether in chloroform followed by evaporation of the solvent in vacuum (Figure 3).

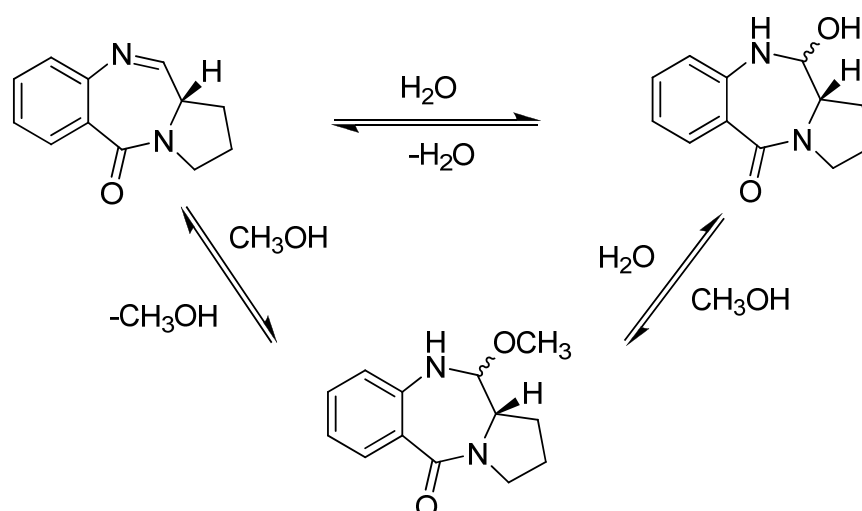


Fig. 3. Carbinolamine-methylether-imine interconversions in the PBDs.

The mechanism of action of the PBDs is associated with their ability to form, an adduct in the minor groove, thus interfering with DNA processing. After insertion in the minor groove, an aminal bond is formed through nucleophilic attack of the N2 of a guanine base at the electrophilic C11 position of PBD. X-Ray diffraction studies on crystals of anthramycin methylethers have shown that the molecule is twisted 0-50° from one end to the other along the axis, and this might fit into one of the grooves of DNA. In the CPK models, the drug fits snugly within the narrow groove without distortion of the DNA helix. The structure of the anthramycin DNA adduct was initially studied independently by Hurley and Kohn using indirect techniques,^[24] but more recently fluorescence spectroscopy, high field NMR and molecular modeling have been employed (Figure 4).

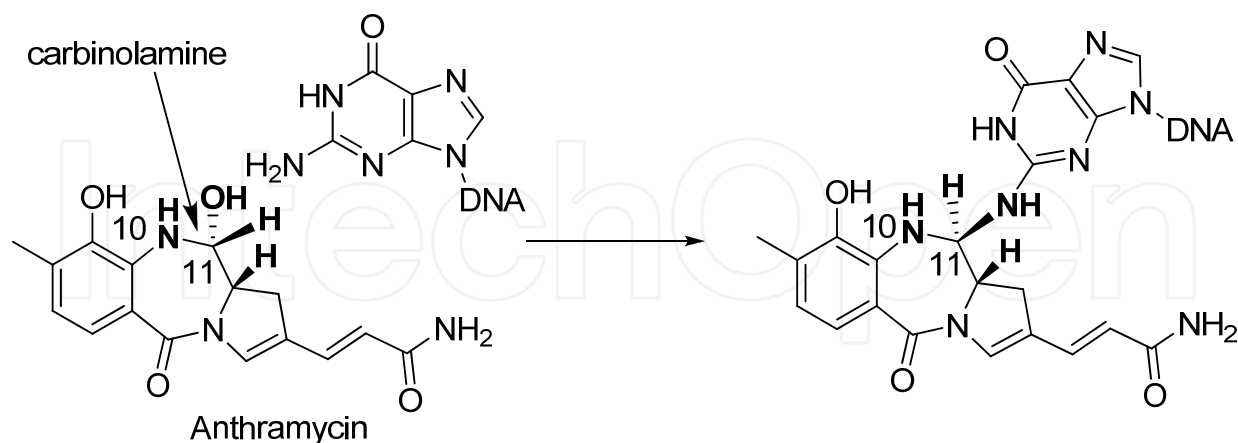
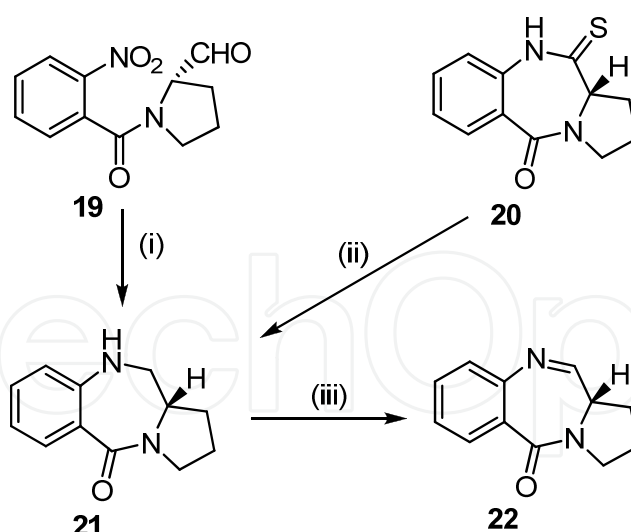


Fig. 4. Proposed mechanism for formation of the anthramycin deoxyguanosine adduct in DNA, showing formation of an amination bond between the C11 position of the PBD and exocyclic N2 of guanine base.

2.3 Preparation of PBDs

Biosynthesis of the naturally occurring PBDs have been extensively elucidated by Hurley and co-workers. The first total synthesis of a carbinolamine containing PBD of anthramycin has been reported by Leimgruber in 1968.^[25] Extensive reviews of the synthetic literature of the PBDs have appeared in 1994, 1998 and 2002.^[26] Various approaches to the synthesis of PBD antibiotics have been investigated, including hydride reduction of seven-membered cyclic dilactams,^[27] reductive cyclization of acyclic nitroaldehydes,^[28] iminothioether approach^[29] cyclization of aminothioacetals,^[30] deprotective cyclization of the diethylthioacetals *via* N10 protected precursors,^[31] oxidation of cyclic secondary amines,^[32] reductive cyclizations^[33] and solid phase approaches.^[34] Various synthetic methods for the synthesis of the PBDs have been reviewed extensively. The N10-C11 carbinolamine, or its chemical equivalent, is a prerequisite for antitumour activity. Several research groups have designed new PBDs with potential DNA binding affinity.^[35-39]

A novel method for the oxidation of PBD secondary amine to the corresponding imines is developed in this laboratory³². Although PBDs with either a secondary amine or amide functionality at N10-C11 are readily synthesized, the introduction of imine or carbinolamine at this position is problematic due to the reactivity of these functional groups. As described in the literature, the cyclic secondary amine precursors have been readily prepared from corresponding nitroaldehydes. This upon oxidation with DMSO/(COCl)₂ or TPAP (tetra-*n*-propyl ammonium perruthenate) gives the corresponding imines in good yields (Scheme 1). The same group has carried out another interesting study on the enzymatic reduction of aryl azides to aryl amines by employing baker's yeast. This biocatalytic reductive methodology has been applied to the chemoenzymatic synthesis of PBDs *via* the reductive cyclization of arylazido aldehydes.^[33,40]



Scheme 1. Reagents and conditions: (i) Pd/C; (ii) Raney Ni; (iii) Swern/TPAP.

3. Structure activity relationship studies

The naturally occurring PBDs namely anthramycin, tomaymycin, sibiromycin, neothramycin and DC-81 have different type of substitutions. The electron-donating substituents are required in the aromatic A ring for biological activity. Bulky substituent like a sugar moiety at C7 position enhances the DNA binding affinity and cytotoxicity. It is interesting to note that C ring modified PBDs appear to provide both greater differential thermal stabilization of DNA duplex and significantly enhance kinetic reactivity during covalent adduct formation. Similarly, the C2-substituted naturally occurring PBDs exhibit more cytotoxicity compared to their unsubstituted counter parts as shown in Figure 5. Based on these considerations a structure activity relationship has been derived by Thurston and co-workers.

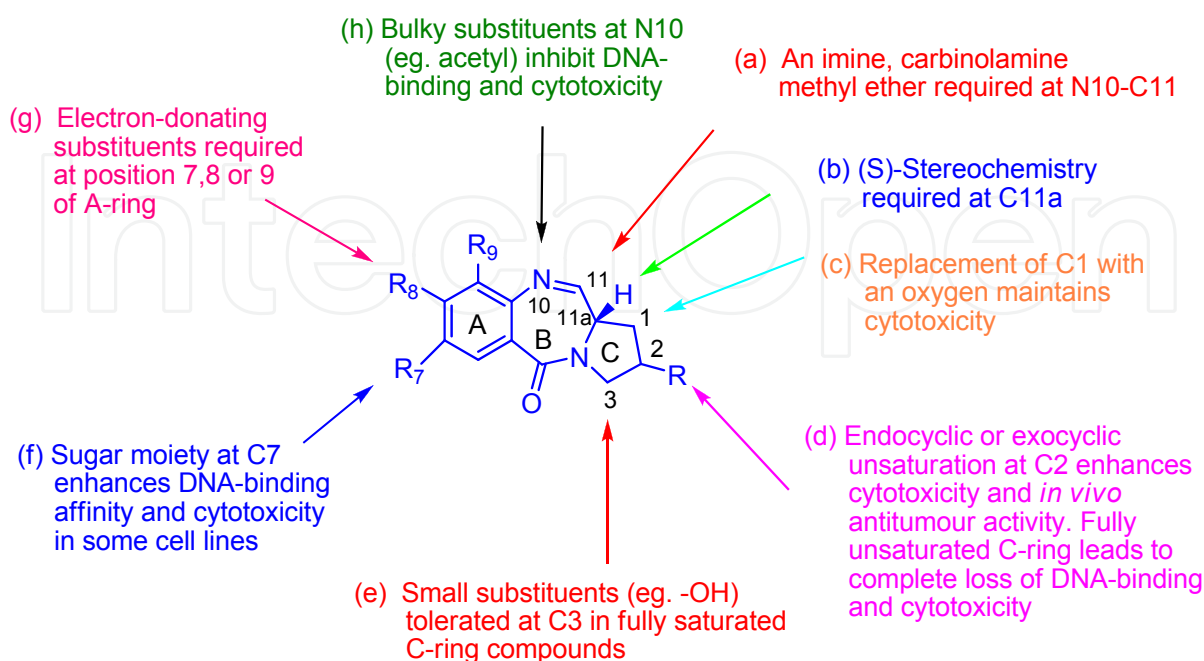
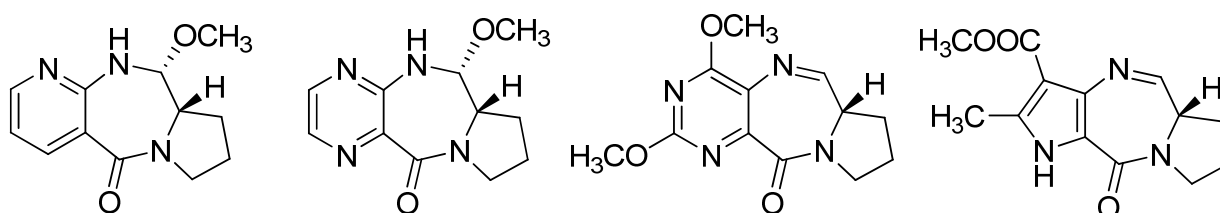


Fig. 5. Structure activity relationship of PBD ring system.

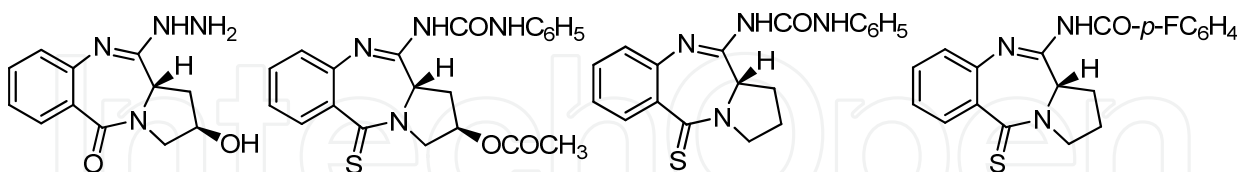
3.1 PBD A-ring modifications

Baraldi and co-workers^[41] have investigated heterocyclic analogues of pyrrolo-[2,1-c][1,4]benzodiazepine (PBD) by replacing A ring with pyrazolo[4,3-e]-pyrrolo[1,2-c][1,4]diazepinone ring system. Some of these ring pyrazole PBD analogues exhibited interesting profile of cytotoxicity. Similarly, Thurston and co-workers^[42] have synthesized some other A-ring heterocyclic PBDs and evaluated their DNA binding affinity. In this study pyrazine and pyrimidine A-ring analogues of PBDs have been prepared and evaluated for their cytotoxic potential. It is observed that the aromatic A-ring has a modest influence on thermal denaturation of DNA. They have also synthesized some tetracyclic PBD analogues like dioxolo[4,5-*h*]-pyrrolo[2,1-c]benzodiazepine-5-one and dieoxano[2,3-*h*]pyrrolo[2,1-c]benzodiazepine-5-one and they have observed that the addition of dioxazole or dioxazine rings to existing A ring of DC-81 significantly reduces DNA binding affinity ($\Delta T_m = 0.1$ to 0.5 °C) and cytotoxicity [$IC_{50} = 0.26$ - 3.4 μ M] compared to DC-81.



3.2 B-ring modifications

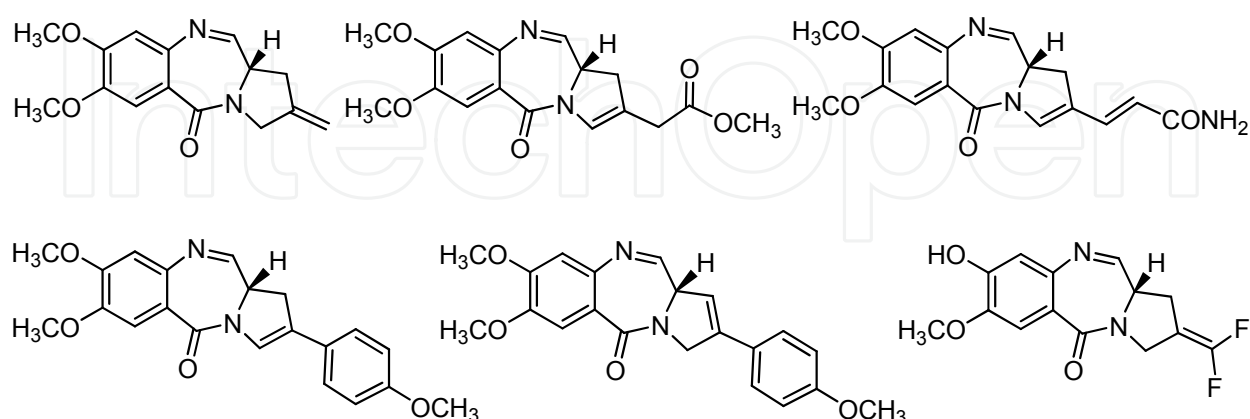
There are reports on B-ring modifications and one of them describes the synthesis and antitumour activity of pyrrolo[2,1-c][1,4]benzodiazepine derivatives and these compounds exhibit moderate antitumour activity. To investigate the role played by the non-covalent interactions Robba and co-workers^[43] have synthesized a series of PBDs having N10-C11 amidine functionality and evaluated the DNA binding through thermal denaturation studies. It is observed that some of these compounds cause a significant increase in melting of calf thymus DNA comparable to the naturally occurring DC-81.



3.3 C-ring modifications

A number of naturally occurring PBDs namely anthramycin, tomaymycin, sibiromycin and neothramycin have different type of substitutions in the C ring. It is interesting to note that these C ring modified PBDs appear to provide both greater differential thermal stabilization of DNA duplex and significantly enhance kinetic reactivity during covalent adduct formation. Similarly, the C2 substituted naturally occurring PBDs exhibit more cytotoxicity compared to their unsubstituted counter parts. Thurston and co-workers^[44] have synthesized a series of C2-*exo* unsaturated PBDs, and C2-C3-*endo* unsaturated PBDs C-ring enhances both DNA-binding reactivity and *in vitro* cytotoxic potency. Recently the same group has reported the synthesis of novel C2-aryl 1,2/2,3-*endo* unsaturated

pyrrolobenzodiazepines as potential antitumour agents and synthesized novel C2-C3 unsaturated PBD analogues containing conjugated acrylyl C2 substituents, these analogues possess not only significant cytotoxicity (in the NCI 60-cell line screen with surprisingly potency equivalent to anthramycin) but also better DNA binding ability.^[45]



Recently, a series of C2-fluorinated PBDs^[46] have synthesized and they have been screened for *in vitro* cytotoxicity against a number of cancer cell lines. The C2-fluorinated PBDs significantly increase the thermal stability of the calf thymus DNA duplex and also these compounds show a 550 fold increase in activity against the CH1 cell line when compared to the unsubstituted PBDs. A ring substituted C2-fluorinated monomers of PBD and DC-81 dimers have also been synthesized in this laboratory.^[47] These compounds have shown good DNA binding ability when compared to A-ring unsubstituted C2-fluorine compounds. Moreover, such fluorinated compounds possess *in vitro* anticancer activity in a number of human cancer cell lines (Figure 6).

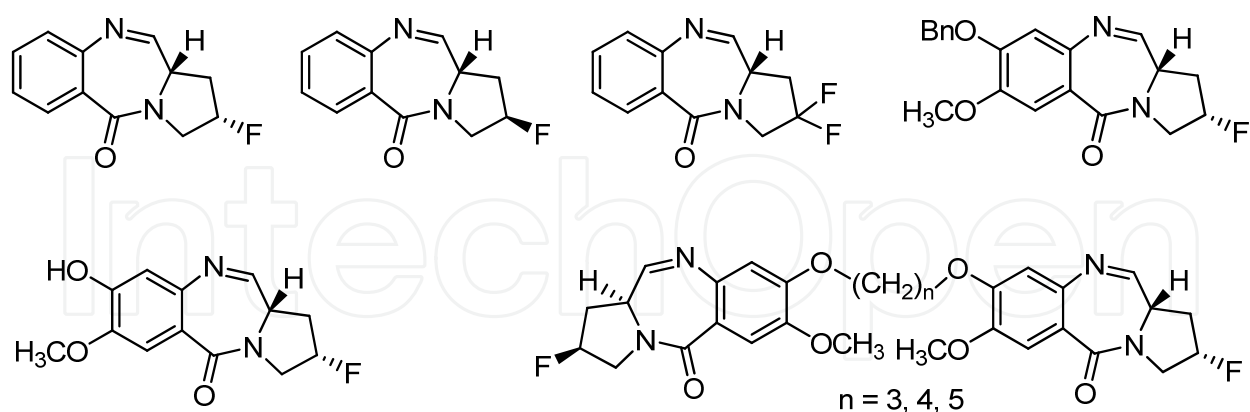


Fig. 6. Structure of fluorinated PBD monomer derivatives shown in a significant increase in melting for calf thymus dna comparable to the naturally occurring DC-81

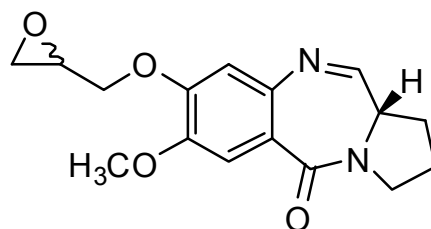
3.4 PBD conjugates

The research of antineoplastic agents is based on the fact that a single molecule of PBD exhibits higher DNA binding affinity as well as more anticancer activity, if it contains more than one pharmacophore, each with different mode of action and capable of recognizing

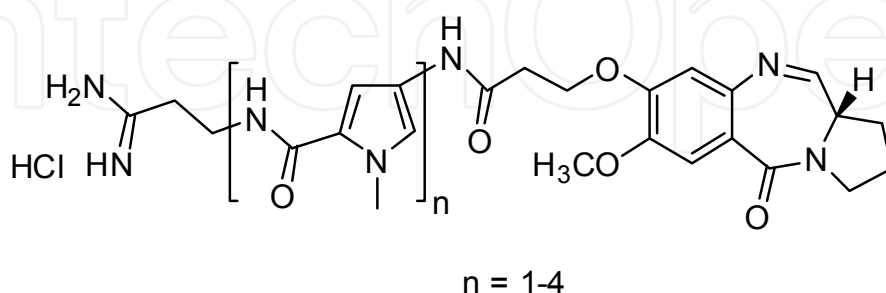
heterogeneous DNA sequences. For this reason, several research groups developed new PBD conjugates, such as cyclopropylbenzindole, distamycin, and netropsin. Synthesis of PBD conjugates has been carried out employing mainly pyrrolobenzodiazepine DC-81, that is linked mostly at C8 position to different moieties; rare examples of C2 linkage were described in literature these last years.⁴⁸

The PBDs have also been used as a scaffold to attach ethylenediamine tetraacetic acid (EDTA),^[49] epoxide,^[50] polyamide^[51] and oligopyrrole moieties^[52] leading to novel hybrids of PBD, which have exhibited sequence selective DNA-cleaving and cross-linking properties. EDTA moiety has been linked to the PBD skeleton (DC-81) to study the covalent binding to DNA and its sequence selectivity.^[53] Detailed analysis of the cleavage sites by laser densitometry suggested that the results are best explained by two major modes of binding/cleaving for (EDTA/DC-81)Fe^{II}. The EDTA-PBD conjugate covalently binds to DNA at 5'-PuGpu sequences leading to site specific cleavage.

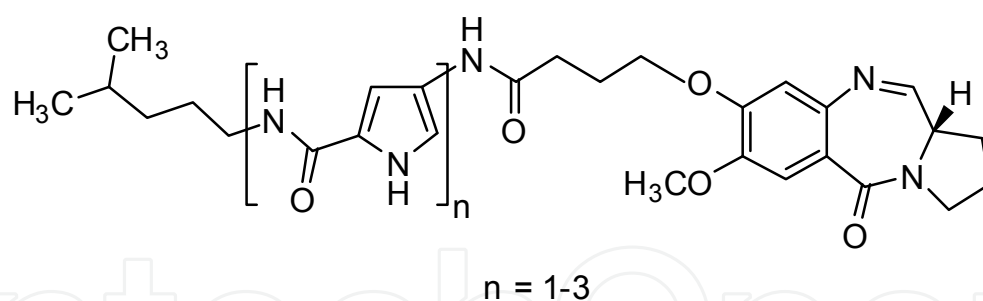
Confalone and co-workers^[54] have synthesized a PBD analogue with an epoxide group substituted at C11a position with the objective of producing a PBD monomer with DNA cross-linking ability, whereas no DNA binding data has been reported. Since attachment at C11a position could sterically hinder the DNA binding, interstrand guanine-guanine cross-linking C8 epoxide containing PBD has been designed and synthesized. It has been considered that the attachment of the epoxide through the C8 position could retain the isohelicity with the contour of the minor groove of the host DNA molecule.^[55]



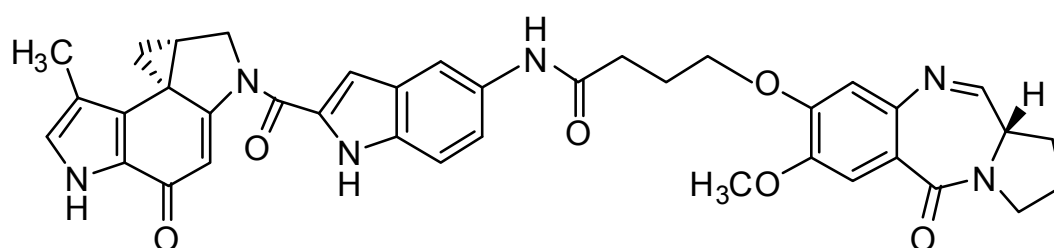
Baraldi and co-workers^[56] have designed and synthesized distamycin-PBD and netropsin-PBD conjugates as novel sequence selective C8-linked PBD hybrids. These hybrids containing 1 to 4 pyrrole units have been investigated for the sequence selectivity and stability of DNA drug complexes.



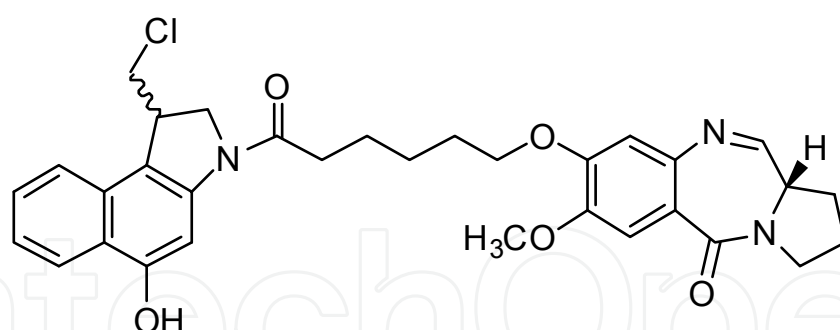
Lown and co-workers^[57] have also reported the synthesis of a series of PBD-lexitropsin conjugates linked through the C8 position with a suitable linker. The conjugation has been achieved by amidic linkage to amine of the lexitropsin unit with the acid moiety of the linker attached to the PBD system. These compounds have been synthesized in view of the effect of their sequence selective binding in DNA duplex.



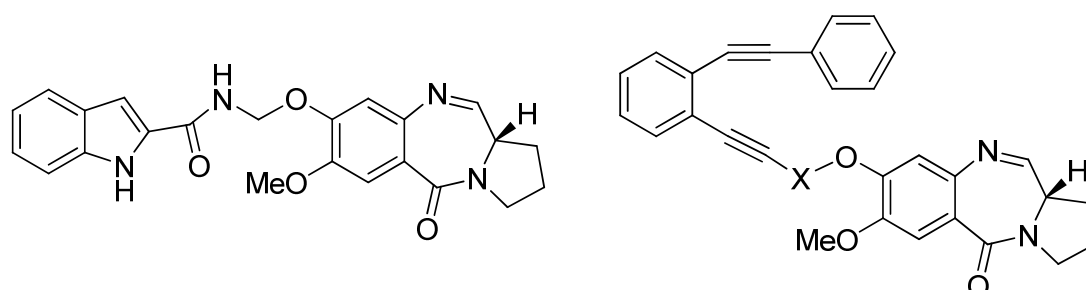
Hurley and co-workers^[58] have synthesized novel DNA-DNA interstrand adenine-guanine cross-linking UTA-6026 compound. Preliminary *in vitro* tests showed that UTA-6026 has remarkable potent cytotoxicity to several tumour cell lines ($IC_{50} = 0.28$ nM in human breast tumour cell line MCF-7, $IC_{50} = 0.047$ nM in colon tumour cell line SW-480 and $IC_{50} = 5.1$ nM in human lung tumour cell line A549).



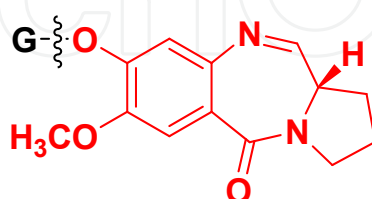
Denny and co-workers^[59] have designed and synthesized unsymmetrical DNA cross-linkers by linking the *seco*-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indo-4-one (*seco*-CBI) to PBD moiety. These compounds have anticipated cross-linking between N3 of adenine and N2 of guanine in the minor groove of DNA.



Recently, Wang and co-workers^[60] have synthesized indole and enediyne PBD conjugates as potential antitumor agents and explained the correlation between antitumor activity and apoptosis.

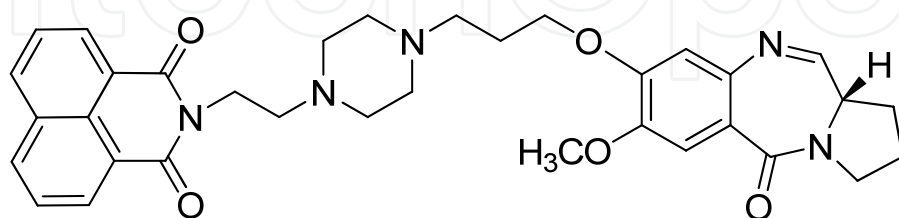


In the past few years, this research group prepared different type of conjugates, in which a known antitumor compound or some simple active moiety tethered to PBD have been designed, synthesized and evaluated for their biological activity (Figure 7).^[61-73] A series of PBD conjugates by linking different DNA interacting ligands such as benzimidazoles, benzothiazoles, naphthalimides, aryl substituted naphalens, chalcones and poly-aromatic hydrocarbons (pyrene amine, chrysene amine and phenanthrinephenyl) by using varying linker length to enhance the DNA binding affinity and antitumour activity. PBD-morpholine, N-methyl piperizine and *N,N*-dimethyl amine hybrids have been prepared in an attempt to improve the water solubility and cytotoxicity of the PBD compounds.

**DC-81**

$$\Delta T_m = 0.3-0.7\text{ }^{\circ}\text{C}$$

The DNA binding ability of these novel PBD conjugates is usually investigated by thermal denaturation studies using *calf thymus* (CT) duplex DNA at pH 7.0 employing the protocols reported in the previous studies.^[67,74] All the PBD conjugates showed better melting temperature of CT-DNA compared to the naturally occurring DC-81. The DNA melting temperature studies indicate that these PBD conjugates exhibit significant DNA binding affinity. The PBD conjugates are effective as DNA binding agents particularly at G-rich sequences.^[75] Additionally when the restriction enzyme digestion assay (RED assay) was carried out for some representative compounds along with the DC-81. This study clearly indicated that most of the conjugates effectively bind at G-rich region of the minor groove of DNA even at lower concentrations. These studies clearly indicate that the linking of the PBD scaffold with other conjugate partners not only enhances the binding potential significantly but also increases the base pair sequence selectivity. With regard to the PBD scaffold it is well established that it forms a covalent linkage at the N2 position of a guanine base of the DNA while the other subunit of this new conjugate is likely to interact within the DNA through the non-covalent interactions.^[63,76] This aspect has been investigated in detail in case PBD-naphthalimide and benzimidazole conjugates examine their sequence selective binding ability.



All the PBD-naphthalimide conjugates also showed promising anticancer activity with GI_{50} values of less than 0.1 to 0.5 μM against human cancer cell lines. One of the conjugate exhibited significant DNA binding affinity, that is the ΔT_m is 25.9 at $0\text{ }^{\circ}\text{C}$. The DNA binding of this conjugate to $d(\text{ACAATTGTT})_2$ was studied by a combination of high-resolution ^1H and ^{31}P 2D NMR spectroscopy and restrained molecular dynamic calculations

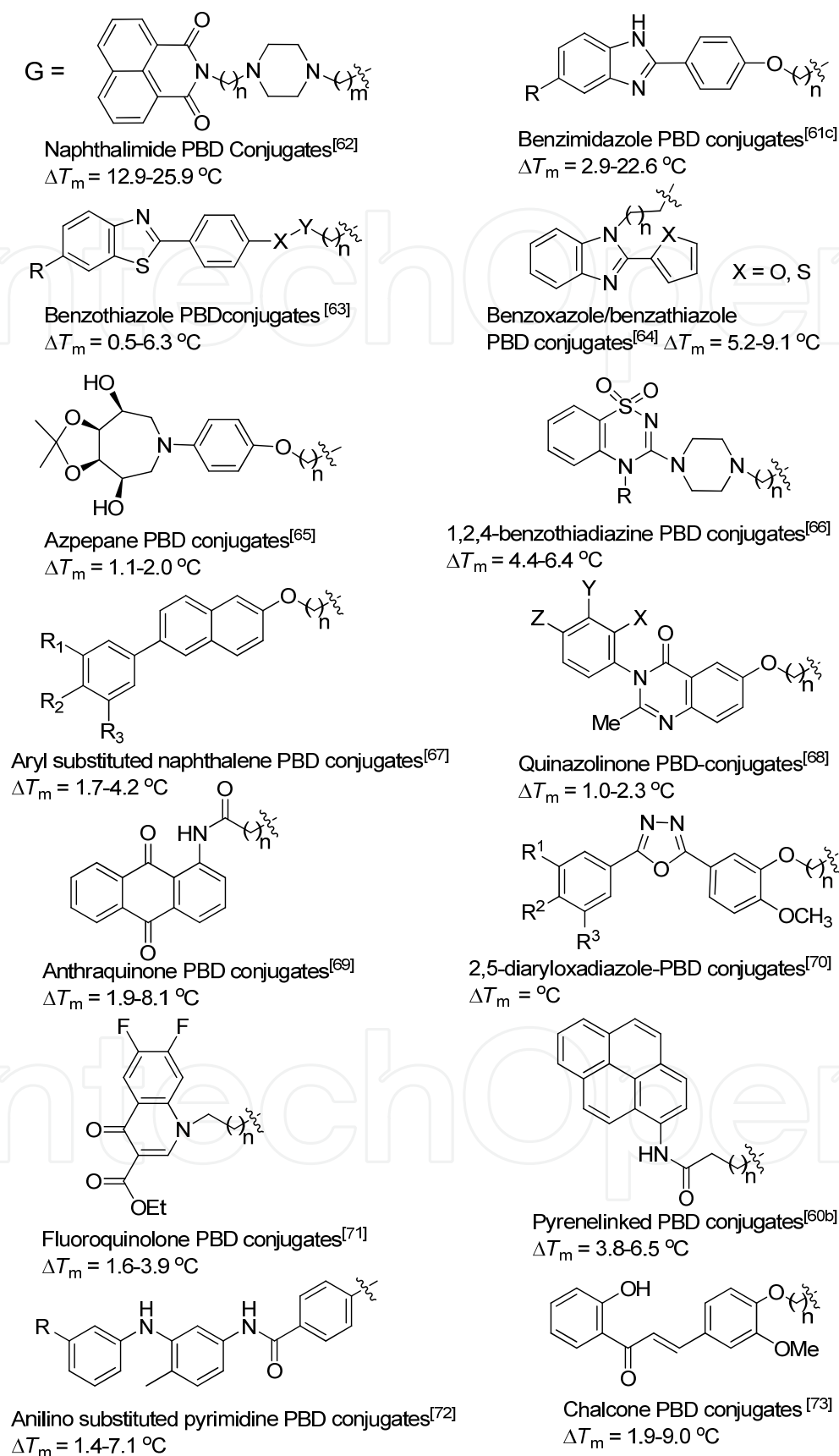


Fig. 7. Some representative PBD conjugates.

in explicit solvent. The bifunctional hybrid binds with its PBD moiety covalently linked within the minor groove to a guanine with an *S* stereochemistry at its covalent linkage site at C11 and a 5'-orientation of its A-ring carrying the linker with the naphthalimide ligand. The latter inserts from the minor groove between an A - A. T - T base pair step resulting in an opposite buckling of the base pairs at the intercalation site and duplex unwinding at adjacent internucleotide steps. There is NMR spectroscopic evidence that the naphthalimide undergoes a ring-flip motion with exchange rates slow to intermediate on the chemical shift time scale at ambient temperatures (Figure 8).^[77]

PBD-benzimidazole conjugates also binds in the DNA minor groove with a preference for (A,T)₄G sequences. Whereas the binding of both ligands is enthalpy-driven and associated with a negative entropy, the benzimidazole hybrid exhibits a less favourable binding enthalpy that is counterbalanced by a more favourable entropic term when compared to the naphthalimide hybrid.^[78]

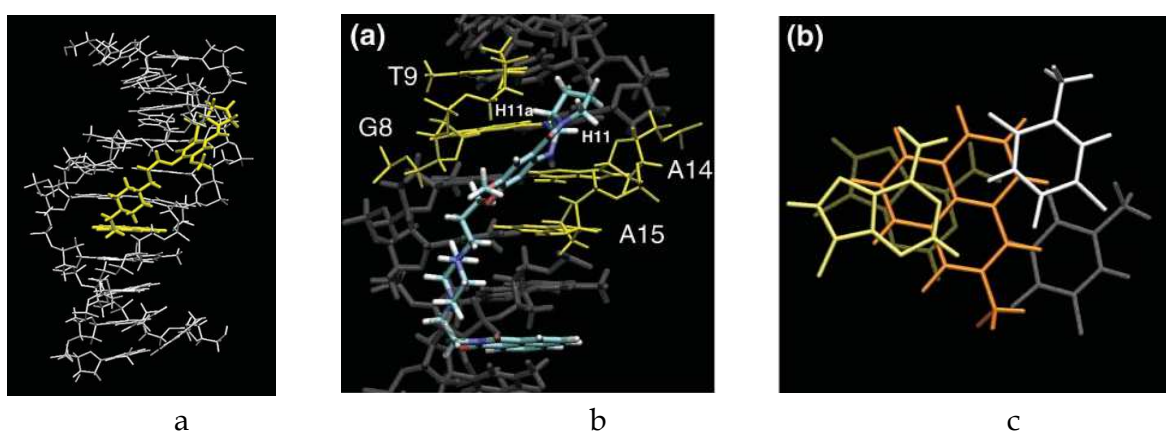


Fig. 8. a. Final energy-minimized structure of the PBD-naphthalimide-(AACAATTGTT)₂ complex; view into the minor groove illustrating the position and orientation of the drug (in yellow) and the naphthalimide intercalation site. b. Close up view into the minor groove of the PBD-naphthalimide-(AACAATTGTT)₂ complex residues G8 and T9 as well as A14 and A15 positioned on the H11a and H11 side of the covalently bound (11s, 11aS) PBD moiety are highlighted. c. Top view of the intercalation site with the naphthalimide, adenine and thymine bases colored in orange, yellow and white, respectively; base pair A5 T16 shown on top.

4. PBD dimers and trimers

A very original and interesting development for the PBD alkylating agents was obtained upon synthesis of bifunctional compounds. Suggs and co-workers have linked the two PBD moieties through the A-ring at C7/A-C7' positions by alkanediyl dioxy linker (**1**) and investigated the DNA binding properties. These molecules have been rationally designed as DNA cross-linkers and they bind reversibly to DNA and protect DNA against the action of certain restriction enzymes.^[79,80] Lown and co-workers have designed and synthesized PBD dimers, joined tail to tail (C-ring) at C2 position through alkylamido linker unit (**2**). A series of these dimers have been evaluated for their cytotoxicity against 9 panels containing 60 human cell lines. It is observed that these compounds exhibited moderate to promising cytotoxic potency against different cancer cells,^[81] some of the dimers are shown in Figure 9.

Thurston and co-workers have synthesized homologous series of C8 diether-linked PBD dimers (**3**, DSB-120) that span approximately six base pairs of DNA and in which sequence selectivity also increased (e.g., purine-GATC-pyrimidine).^[82,83] DSB-120 exhibits potent *in vitro* cytotoxicity and enhances DNA binding affinity and sequence specificity as compared to the natural occurring DC-81. This improvement in biological activity has been attributed to the ability of these compounds to link to DNA irreversibly via guanine residues on opposite strands because of the presence of two active sites (i.e., two imine functionalities).^[84] The *in vivo* studies of this compound were not encouraging, and the low therapeutic index observed was partly due to the reaction of this compound with cellular thiol-containing molecules before reacting at the tumor site.^[85] Recently, another new cross-linking PBD dimer (**4**, SJG-136) having C2/C2'-exo unsaturation (that exhibits high DNA binding affinity) has been prepared by the same group.^[86] This investigation has highlighted the effect of C2 unsaturation on the *in vitro* and *in vivo* cytotoxic activity. Interestingly, the comparison of this PBD dimer with its tetralactam analogue demonstrates that for maximum cytotoxicity an electrophilic imine or carbinolamine moiety is essential at the N10-C11 position of the PBD units.

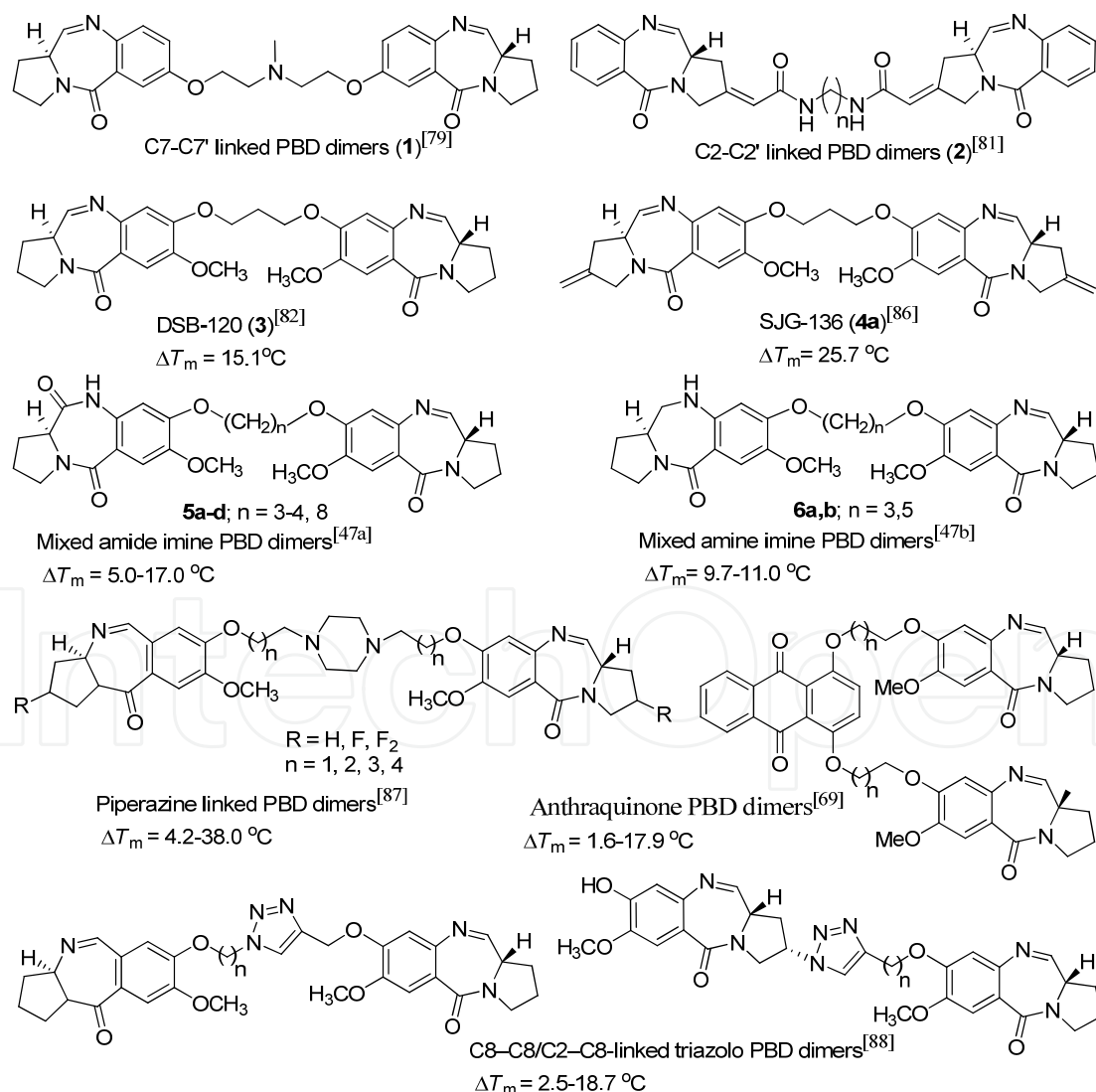


Fig. 9. Some of the PBD dimers.

SJG-136 (**4**) is a sequence-selective DNA-interactive agent that is about to enter phase II clinical trials for the treatment of malignant disease. Previous studies on the pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) dimers, typified by SJG-136 and DSB-120 (**3**), have shown that these planar ligands react with the exocyclic NH₂ groups of two guanine bases in the base of the minor groove of DNA to form an irreversible interstrand cross-linked sequence-specific adduct. Using high-field NMR, we have characterized and modeled the previously predicted interstrand duplex adduct formed by SJG-136 with the self-complementary 5'-d(CICGATCICG)₂ duplex (**4**). This SJG-136 NMR-refined adduct structure has been compared with previous high-field NMR studies of the adducts of the closely related PBD dimer DSB-120 with the same duplex and of the adduct of tomaymycin formed with 5'-d(ATGCAT)₂. Surprisingly, the SJG-136 duplex adduct appears to be more closely related to the tomaymycin adduct than to that of DSB-120 adduct with respect to the orientation and depth of insertion of the ligand within the minor groove. The intrastrand duplex adduct formed in the reaction of SJG-136 with the noncomplementary 5'-d(CTCATCAC)·(GTGATGAG) duplex (**4**) has also been synthesized and modeled. In this duplex adduct, the nature of the cross-link was confirmed, the central guanines were identified as the sites of alkylation, and the stereochemical configuration at C11 at both ends of the SJG-136 molecule was determined to be *S*. The NMR-refined solution structures produced for the intrastrand adduct confirm the previously proposed structure (which was based solely on mass spectroscopy). Both the inter and intrastrand SJG-136 duplex adducts form with minimal distortion of the DNA duplex (Figure 10). These observations have an impact on the proposal for the mechanism of action of SJG-136 both *in vitro* and *in vivo*, on the repair of its adducts and mechanism of resistance in cells, and, potentially, on the type of pharmacodynamic assay to be used in clinical trials.^[89-93]

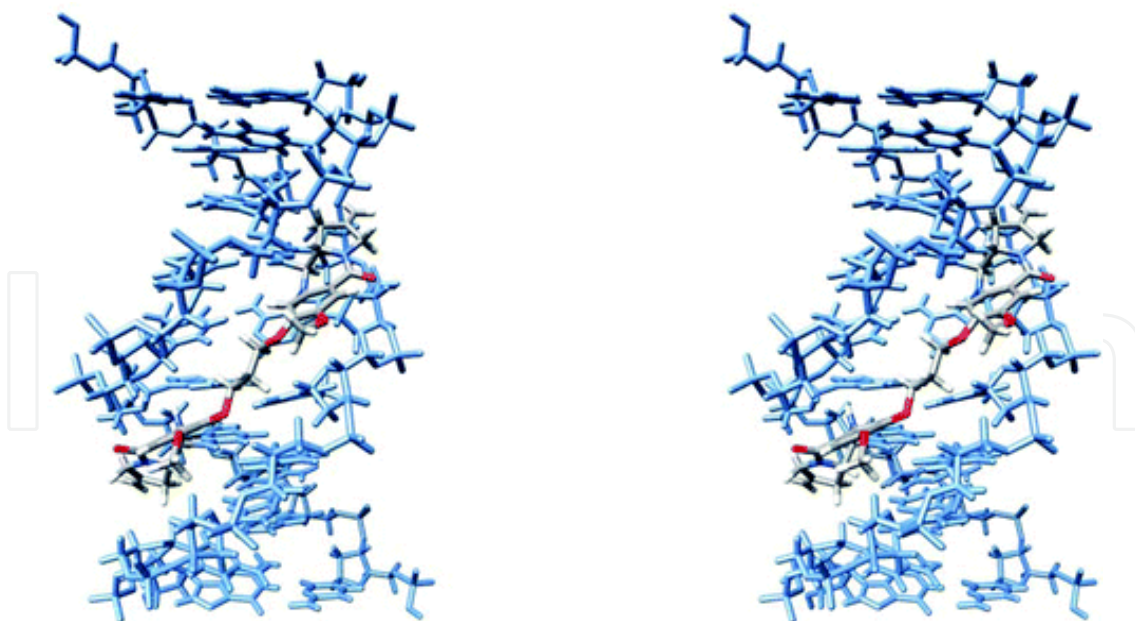


Fig. 10. Stereoview of the 5'-d(CICGATCICG)₂-SJG-136 intrastrand adduct. DNA strands are colored blue, and SJG-136(**4**) is shown in atom colors. Watson-Crick base pairing has been maintained, and there is minimal distortion to the β -helical structure of the DNA backbone. Models were produced in the SYBYL modeling suite and images produced using UCSF Chimera.^[94]

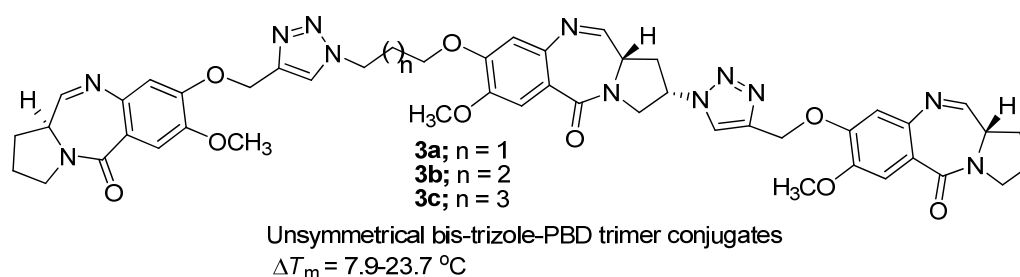
4.1 Noncross-linking PBD dimers

This laboratory has been involved in the structural modifications of the PBD ring system and development of new synthetic strategies. It is been observed in the literature that no effort has been made to prepare and investigate PBD dimers with one imine functionality alone for exploring their cytotoxicity as noncross-linking agents (**5a-d** and **6a,b**). These type of PBD dimers were prepared to understand the contributions from the non-covalent interactions by one of the subunit in such dimers. It was observed that by incorporation of a non-covalent component surprising the DNA-binding affinity significantly enhances in such mixed type of PBD dimers.^[47] One of these dimer with five alkane spacer (**5c**) elevates the helix melting temperature of CT-DNA remarkably to 17.0 °C after incubation for 18 h at 37 °C.

The binding affinity of the compounds was also measured by restriction endonuclease digestion assay based on inhibition of the restriction endonuclease *Bam*HI. This study reveals the significance of noncovalent interactions in combination with covalent bonding aspects when two moieties of structural similarities are joined together. This allows the mixed imine-amide PBD dimer with a five carbon chain linker to achieve an isohelical fit within the DNA minor groove taking into account both the covalent bonding and the noncovalent binding components. This has been supported by binding studies (Figure 11), which indicate that the PBD dimer with a five carbon chain linker (**5c**) gives rise to maximum stabilization of the complex with DNA at the minor groove as compared to the other PBD dimers with three (**5a**), four (**5b**) and eight (**5d**) carbon chain linkers. The energy of interaction for all of the complexes studied was in correlation with the ΔT_m values. Mixed imine-amine pyrrolobenzodiazepine (PBD) dimers that are comprise of a DC-81 and secondary amine (N10) of DC-81 subunits tethered to their C8 positions through alkanedioxy linkers (comprised of three and five carbons) was also studied. These noncross-linking unsymmetrical PBD dimers exhibit significant DNA minor groove binding ability and one of them that was **6b** linked through the pentanedioxy chain exhibits efficient DNA binding ability ($\Delta T_m = 11.0$ °C) in compared to naturally occurring DC-81, ($\Delta T_m = 0.7$ °C).

4.2 PBD trimers

The unsymmetricalbis-1,2,3-triazolo-PBD trimers have been designed and synthesized by employing 'click' chemistry process. Interestingly, by using this 'click' chemistry protocol the solubility aspects have been improved that facilitated the purification and isolation of the target compounds. These new PBD trimers have shown significant DNA-binding ability. Molecular modelling studies substantiate the formation of three covalent bonds with the PBD trimer and guanine. One of the representative compound **3c** appears to be the optimal binder as further increase in linker or chain length decreases the binding strength of these compounds with DNA (Figure 12).^[95]



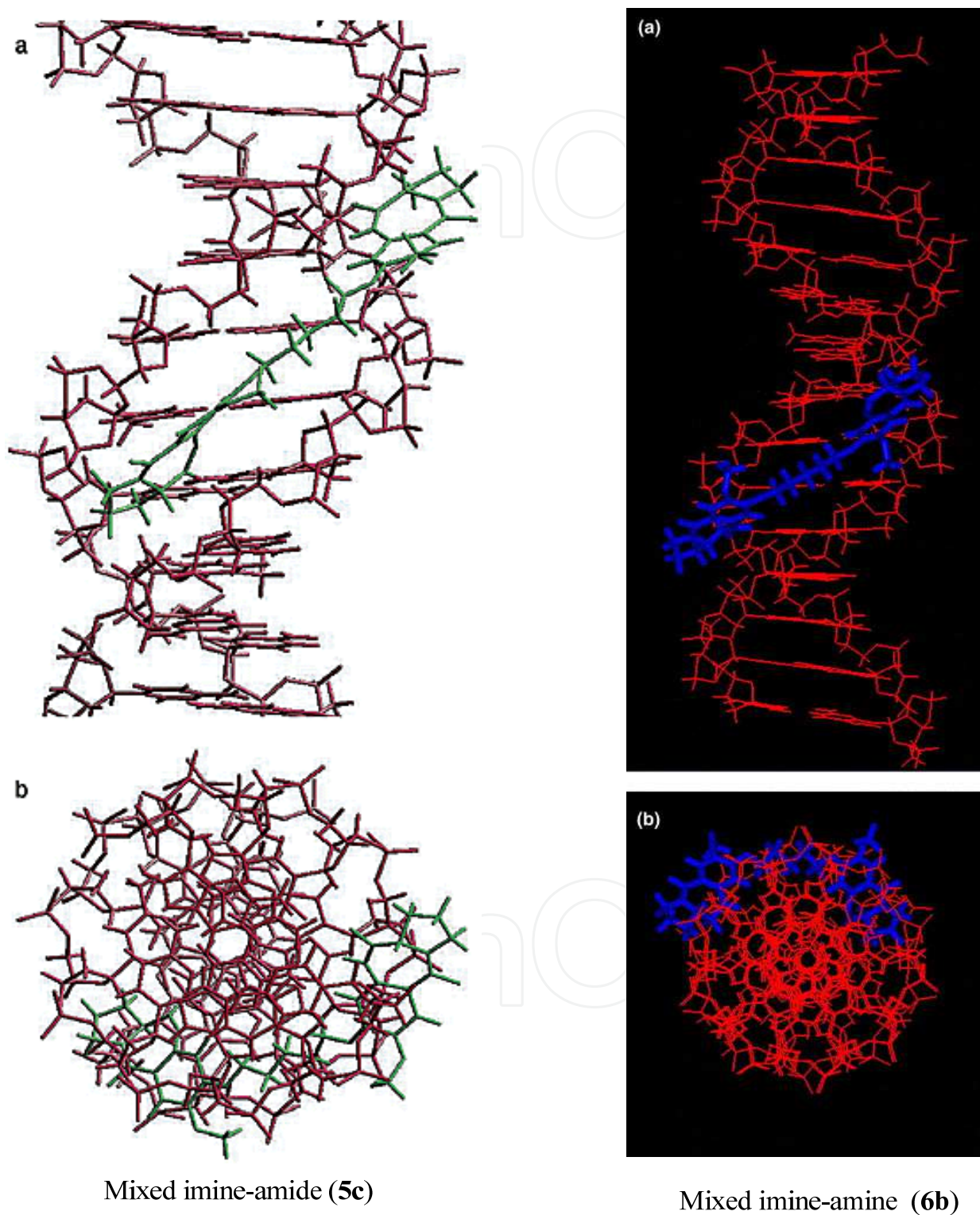


Fig. 11. Projection diagram showing the DNA-5c and 5b complexes. (a) Side on view and (b) down the helix axis.

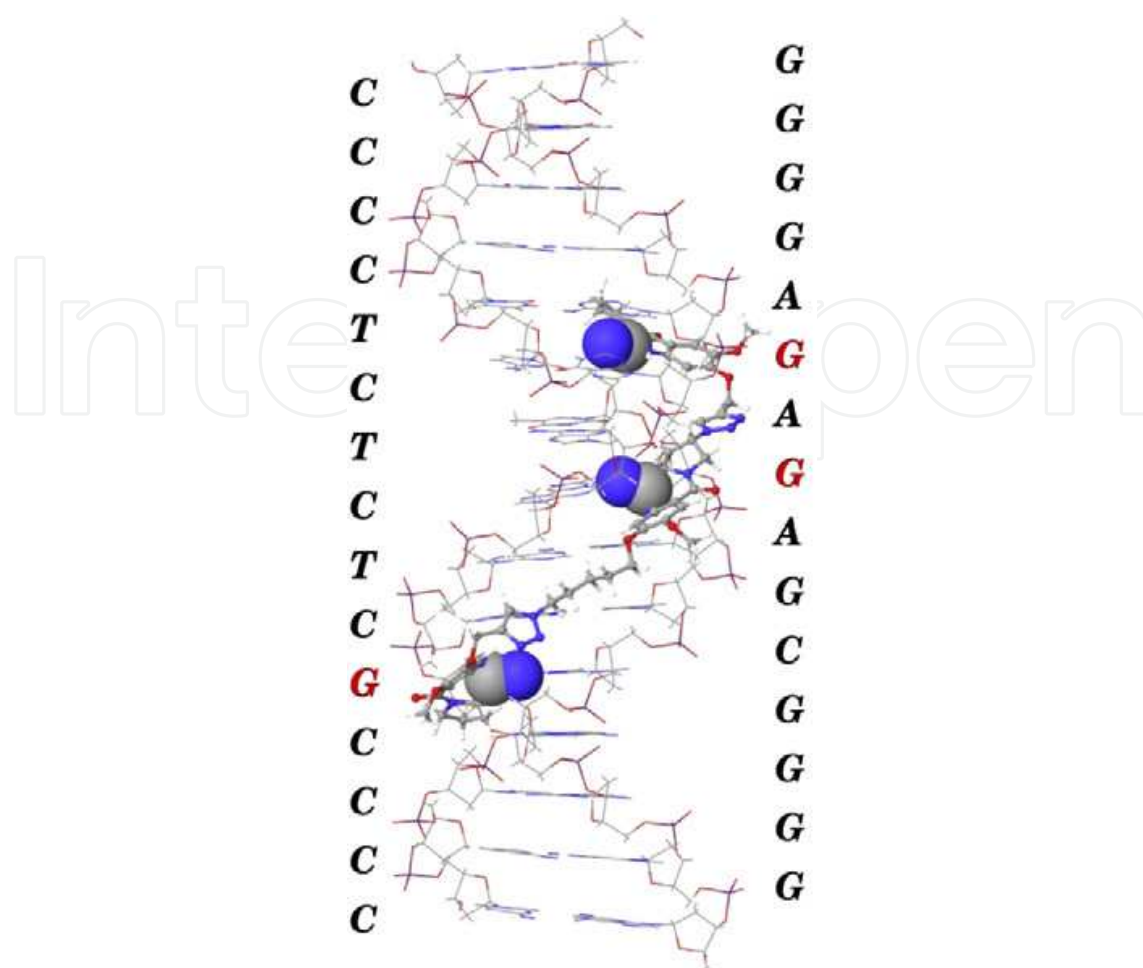


Fig. 12. Covalent bonding of PBD trimer **3c** with DNA (guanine residues involved in the bonding are written in red color, C-atom of PBD and N-atom of guanine are shown in CPK).

5. Concluding remarks

In conclusion, synthesis of imine containing pyrrolobenzodiazepines (PBDs) has often posed practical problems towards its isolation and preparation. Based on the biological importance of these pyrrolobenzodiazepines (PBDs) enhance the selectivity as well as anticancer activity. The design of hybrid ligands has provided a basis for modulating the sequence-selective binding behavior and/or tailoring the hybrid ligands for mixed-sequence recognition. This has also allowed to demonstrate that the design of such hybrids enhances anticancer activity as well as stability of drug-DNA complexes. Further some of these compounds exhibiting apoptosis inducing ability. Some of the new compounds, PBD-benzimidazole hybrids and piperazine-linked PBD dimers are undergoing preclinical studies. SJG-136 is currently undergoing Phase II evaluation in both the United States (through the NCI) and United Kingdom (through Cancer Research United Kingdom). The design of mixed dimers has allowed to illustrate the important role played by the non-covalent interactions in the enhancement of DNA-binding affinity. Interestingly, new PBD trimers have shown significant DNA-binding ability, binding and molecular docking studies substantiate the formation of three covalent bonds with the PBD trimer and guanine. To date, a large number of PBD best molecules have been synthesized, highlighting that this

area of research is extremely important for achieving considerable importance in the recognition of DNA sequences.

However, this search for new molecules with enhanced selectivity is in progress in order to recognise about 15 bp for DNA sequences within the human genome.

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7. References

- [1] (a) Burke, B. M.; Wilkes, G. M.; Ingwersen, K. C. *Cancer chemotherapy. Published by Jones and Bartlett publishers*, 2001, 1; (b) Sarter, B.; Burke, B. M.; Wilkes, G. M.; Ingwersen, K. C. *Cancer chemotherapy care plans handbook, Published by Jones and Bartlett publishers*, 2001, 1.
- [2] (a) Cech, T. R. *Annu. Rev. Biochem.* 1990, 59, 543; (b) Michel, F.; Ferat, J. L. *Annu. Rev. Biochem.* 1995, 64, 435; (c) Cech, T. R.; Gesteland, R. F.; Atkins, J. F. *Cold Spring Harbour New York*, 1993; 239.
- [3] Wong, A. H. J.; Wigley, G. J.; Kolpak, F. J.; Crawford, J. L.; Van Boom, G.; Marel, V.; Reich, A. *Nature* 1979, 282, 680.
- [4] (a) Hsiang, Y. H.; Jiang, T. B.; Lieu, L. F. *Mol. Pharmacol.* 1989, 36, 371; (b) Pomnier, Y.; Kohn, K. W. F. L.; *CRC Pre. Inc*, 1989, 175; (c) Yamashita, Y.; Kawada, S. Z.; Nakano, H. *Biochemistry* 1991, 30, 5838; (d) Liu, L. F. *Annu. Rev. Biochem.* 1989 58, 351; (e) Franklin, R. E.; Gosting, R. G. *Trans. Faraday Soc.* 1954, 50, 298.
- [5] Courtney, S. M.; Thurston, D. E. *Tetrahedron Lett.* 1993, 34, 5327.
- [6] Dwyer, P. J.; Shoemaker, D.; Zaharko, D. S.; Grieshaber, C.; Plowman, *Cancer Chemother. Pharmacol.* 1987, 19, 6.
- [7] Zimmur, C. *Prog. Nucleic Acid Res. Mol. Biol.* 1975, 15, 285.
- [8] Wartell, R. N.; Larson, J. E.; Wells, R. E. *J. Biol. Chem.* 1974, 249, 6719.
- [9] Karlovsky, P.; Decock, A. W. *Anal Biochem.* 1991, 194, 192.
- [10] (a) Kunimoto, S.; Masuda, T.; Kanbayashi, M.; Hamada, M.; Umazawa, H. *J. Antibiot.* 1980, 33, 665; (b) Takeuchi, T.; Miyamoto, M.; Ishizuka, M.; Naganawa, H.; Kondo, S.; Hamada, M.; Umezawa, H. *J. Antibiot.* 1976, 29, 93.
- [11] Shimizu, K. I.; Kawamoto, I.; Tomita, F.; Morimoto, M.; Fuzimoto, K. *J. Antibiot.* 1982, 35, 972.
- [12] (a) Hara, M.; Tamaoki, T.; Yashida, M.; Morimoto, M.; Xlakano, J. *Antibiot.* 1988, 41, 702; (b) Langley, D. R.; Thurston, D. E. *J. Org. Chem.* 1987, 52, 91.
- [13] (a) Itoh, J.; Watanabe, H. O.; Ishii, S.; Gomi, S.; Nagasawa, M. and Yamamoto, A. *J. Antibiot.* 1988, 41, 1281; (b) Leber, J. D.; Hooper, J. R. C.; Holden, K. G.; Johnson, R. R.; Hed, S. M. *J. Am. Chem. Soc.* 1988, 110, 299.
- [14] Hurley, L. H.; Reck, T.; Thurston, D. E.; Langley, D. R.; Holder, K. G.; Hertzberg, R. P.; Hwover, J. R. E.; Gallegher, G.; Faucette, L. F. Jr.; Mong, S. M.; Johnson, R. K. *Chem. Res. Toxicol.* 1988, 1, 258.
- [15] Arima, K.; Kohsaka, M.; Tamura, G.; Imanaka, H.; Sakai, H. *J. Antibiot.* 1972, 25, 437.

- [16] Konishi, M.; Ohkuma, H.; Naruse, N.; Kawaguchi, H. *J. Antibiot.* 1984, 37, 200.
- [17] Tsunkawa, M.; Kamei, H.; Konishi, M.; Miyaki, T.; Oki, T.; Kawaguchi, H. *J. Antibiot.* 1988, 41, 1366.
- [18] (a) Kunitmoto, M.; Masuda, T.; Kanbayashi, N.; Hamada, M.; Naganawa, H.; Miyamoto, M.; Takeuchi, T.; Unezawa, H. *J. Antibiot.* 1980, 33, 665; (b) Thurston, D. E.; Murthy, V. S.; Langley, D. R.; Jones, G. B. *Synthesis* 1990, 1, 81; (c) Bose, D. S.; Jones, G. B.; Thurston, D. E. *Tetrahedron*, 1992, 48, 751.
- [19] (a) Thurston, D. E. *The Macmillan Press Ltd. London, U. K.* 1993; 54; (b) Petrusek, R. L.; Uhlenhopp, E. L.; Duteau, N.; Hurley, L. H. *J. Biol. Chem.* 1982, 257, 6207.
- [20] Boyd, F. L.; Stewart, D.; Remers, W. A.; Barkley, M. D.; Hurley, L. H. *Biochemistry* 1990, 29, 2387.
- [21] Puvvada, M. S.; Hartley, J. A.; Jenkins, T. C.; Thurston, D. E. *Nucleic Acids Res.* 1993, 21, 3671.
- [22] Puvvada, M. S.; Forrow, S. A.; Hartley, J. A.; Stephenson, P.; Gibson, I.; Jenkins, T. C.; Thurston, D. E. *Biochemistry* 1997, 36, 2478.
- [23] Kopka, M. L.; Goodsell, D. S.; Baikalov, I.; Grzeskowiak, K.; Cascio, D.; Dickerson, R. E. *Biochemistry* 1994, 33, 13593.
- [24] (a) Kunitmoto, S.; Masuda, T.; Kanbayashi, N.; Hamada, M.; Nayanawa, H.; Miyamoto, M.; Takeuchi, T.; Unezawa, H. *J. Antibiot.* 1980, 33, 665; (b) Kohn, K. W.; Speous, C. L. *J. Mol. Biol.* 1970, 5, 551; (c) Kohn, K. W.; Gloubiger, D.; Zmijewski, M. *Biochem. Biophys. Acta.* 1974, 361, 228; (d) Hurley, L. H.; Gairpla, C.; Smijewski, M. *Biochem. Biophys. Acta.* 1977, 475, 521; (e) Kaplan, D. J.; Hurley, L. H. *Biochemistry* 1981, 20, 7572; (f) Petrusek, R. L. *J. Biol. Chem.* 1981, 257, 6207; (g) Tendler, M. D.; Korman, S. *Nature* 1963, 199, 501.
- [25] Leimgruber, W.; Batcho, A. D.; Czajkowski, R. C. *J. Am. Chem. Soc.* 1968, 90, 5641.
- [26] (a) Thurston, D. E.; Bose, D. S. *Chem. Rev.* 1994, 94, 433; (b) Kamal, A.; Rao, M. V.; Reddy, B. S. N. *Chemistry of Heterocyclic Compounds* 1998, 1588; (c) Kamal, A.; Rao, M. V.; Laxman, N.; Ramesh, G.; Reddy, G. S. K. *Current Medicinal Chemistry–Anti-Cancer Agents* 2002, 2, 215.
- [27] (a) Kaneko, T.; Wong, H.; Doyle, T. W. *Tetrahedron Lett.* 1983, 24, 5165; (b) Suggs, J. W.; Wang, Y. S.; Lee, K. S. *Tetrahedron Lett.* 1985, 26, 4871.
- [28] Lown, J. W.; Joshua, A. V. *Biochem. Pharmacol.* 1979, 28, 2017.
- [29] (a) Langlois, N.; Favre, F.; Rojas, A. *Tetrahedron Lett.* 1993, 34, 4635; (b) Kaneko, T.; Wong, H.; Doyle, T. W.; Rose, W. C.; Bradner, W. T. *J. Med. Chem.* 1985, 28, 388.
- [30] (a) Langley, D. R.; Thurston, D. E. *J. Org. Chem.* 1987, 52, 91; (b) Courtney, S. M.; Thurston, D. E. *Tetrahedron Lett.* 1993, 34, 5327; (c) Bose, D. S.; Jones, G. B.; Thurston, D. E. *Tetrahedron* 1992, 48, 751.
- [31] Wilson, S. C.; Howard, P. W.; Forrow, S. M.; Hartley, J. A.; Adams, L. J.; Jekins, T. C.; Kelland, L. R.; Thurston, D. E. *J. Med. Chem.* 1999, 42, 4028.
- [32] (a) Kamal, A.; Rao, N. V. *Chem. Commun.* 1996, 385; (b) Kamal, A.; Howard, P. W.; Reddy, B. S. N.; Reddy, B. S. P.; Thurston, D. E. *Tetrahedron* 1997, 53, 3223; (c) Kraus, G. A.; Melekhov, A. *Tetrahedron* 1998, 54, 11749.
- [33] Kamal, A.; Laxman, E.; Laxman, N.; Rao, N. V. *Bioorg. Med. Chem. Lett.* 2000, 10, 2311.

- [34] (a) Berry, J. M.; Howard, P. W.; Thurston, D. E. *Tetrahedron Lett.* 2000, 41, 6171; (b) Kamal, A.; Reddy, G. S. K.; Raghavan, S. *Bioorg. Med. Chem. Lett.* 2001, 41, 387.
- [35] Fukuyama, T.; Liu, G.; Linton, S. D.; Lin, S. C.; Nishino, H. *Tetrahedron Lett.* 1993, 34, 2577.
- [36] Miyamoto, M.; Kondo, S.; Naganawa, H.; Maeda, K.; Ohno, M.; Umezawa, H. *J. Antibiot.* 1973, 30, 340.
- [37] Thurston, D. E.; Langley, D. R. *J. Org. Chem.* 1986, 51, 705.
- [38] (a) Kamal, A.; Laxman, E.; Arifuddin, M. *Tetrahedron Lett.* 2000, 41, 7743; (b) Kamal, A.; Laxman, E.; Reddy, P. S. M. M. *Tetrahedron Lett.* 2000, 41, 8631.
- [39] (a) Kamal, A.; Reddy, G. S. K.; Reddy, K. L. *Tetrahedron Lett.* 2001, 42, 6969; (b) Kamal, A.; Reddy, G. S. K.; Reddy, K. L.; Raghavan, S. *Tetrahedron Lett.* 2002, 43, 2103.
- [40] (a) Kamal, A.; Laxman, E.; Arifuddin, M. *Tetrahedron Lett.* 2000, 41, 7743; (b) Kamal, A.; Laxman, E.; Reddy, P. S. M. M. *Tetrahedron Lett.* 2000, 41, 8631.
- [41] Baraldi, P. G.; Leoni, A.; Cacciari, B.; Manfredini, S.; Simoni, D.; Bergomi, M.; Menta, E.; Spinelli, S. *J. Med. Chem.* 1994, 37, 4329.
- [42] Bose, D. S.; Thompson, A. S.; Smellie, M.; Berardini, M. D.; Hartley, J. A.; Jenkins, T. C.; Neidle, S.; Thurston, D. E. *Chem. Commun.* 1992, 1518.
- [43] Foloppe, M. P.; Rault, S.; Thurston, D. E.; Jenkins, T. C.; Robba, M. *Eur. J. Med. Chem.* 1996, 31, 407.
- [44] (a) Gregson, S. J.; Howard, P. W.; Corcoran, K. E.; Barcella, S.; Yasin, M. M.; Hurst, A. A.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *Bioorg. Med. Chem. Lett.* 1999, 10, 1845; (b) Gregson, S. J.; Howard, P. W.; Barcella, S.; Nakamya, A.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *Bioorg. Med. Chem. Lett.* 1999, 10, 1849.
- [45] (a) Cooper, N.; Hagan, D. R.; Tiberghien, A.; Ademefun, T.; Matthews, C. S.; Howard, P. W.; Thurston, D. E. *Chem. Commun.* 2002, 1764; (b) Kang, G. D.; Howard, P. W.; Thurston, D. E. *Chem. Commun.* 2003, 1688; (c) Chen, Z.; Gregson, S. J.; Howard, P. W.; Thurston, D. E. *Bioorg. Med. Chem. Lett.* 2004, 14, 1547.
- [46] O'Neil, I. A.; Thompson, S.; Kalindjian, S. B.; Jenkins, T. C. *Tetrahedron Lett.* 2003, 44, 7809.
- [47] (a) Kamal, A.; Ramesh, G.; Laxman, N.; Ramulu, P.; Srinivas, O.; Neelima, K.; Kondapi, A. K.; Srinu, V. B.; Nagarajaram, H. A. *J. Med. Chem.* 2002, 45, 4679; (b) Kamal, A.; Ramesh, G.; Srinivas, O.; Ramulu, P.; Laxman, N.; Rehana, T.; Deepak, M.; Achary, M. S.; Nagarajaram, H. A. *Bioorg. Med. Chem.* 2004, 12, 5427.
- [48] Antonow, D.; Kaliszczak, M.; Kang, G. D.; Coffils, M.; Tiberghien, A. C.; Cooper, N.; Barata, T.; Heidelberger, S.; James, C. H.; Zloh, M.; Jenkins, T. C.; Reszka, A. P.; Neidle, S.; Guichard, S. M.; Jodrell, D. I.; Hartley, J. A.; Howard, P. W.; Thurston, D. E. *J. Med. Chem.* 2010, 53, 2927.
- [49] Thurston, D. E.; Morris, S. J.; Hartley, J. A. *Chem. Commun.* 1996, 563.
- [50] Wilson, S. C.; Howard, P. W.; Forrow, S. M.; Hartley, J. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *J. Med. Chem.* 1999, 42, 4028.
- [51] Reddy, B. S. P.; Damayanthi, Y.; Reddy, B. S. N.; Lown, W. J. *Anti-Cancer Drug Des.* 2000, 15, 225.

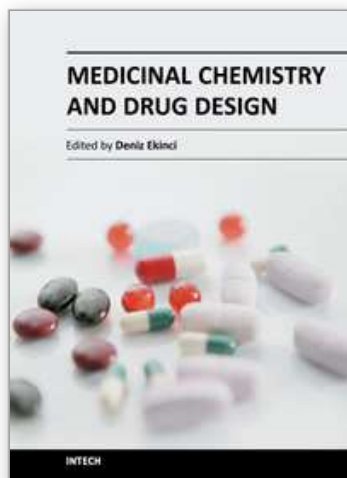
- [52] Baraldi, P. G.; Balboni, G.; Cacciari, B.; Guiotto, A.; Manfredini, S.; Romagnoli, R.; Spalluto, G.; Thurston, D. E.; Howard, P. W.; Bianchi, N.; Rutigliano, C.; Mischiati, C.; Gambari, R. *J. Med. Chem.* 1999, 42, 5131.
- [53] Thurston, D. E.; Morris, S. J.; Hartley, J. A. *Chem. Commun.* 1996, 563.
- [54] Confalone, P. N.; Huie, E. M.; Ko, S. S.; Cole, G. M. *J. Org. Chem.* 1988, 53, 482.
- [55] Wilson, S. C.; Howard, P. W.; Thurston, D. E. *Tetrahedron. Lett.* 1995, 36, 6333.
- [56] Baraldi, P. G.; Balboni, G.; Cacciari, B.; Guiotto, A.; Manfredini, S.; Romagnoli, R.; Spalluto, G.; Thurston, D. E.; Howard, P. W.; Bianchi, N.; Rutigliano, C.; Mischiati, C.; Gambari, R. *J. Med. Chem.* 1999, 42, 5131.
- [57] Reddy, B. S. P.; Damayanthi, Y.; Reddy, B. S. N.; Lown, J. W. *Anti-Cancer Drug Design* 2000, 15, 225.
- [58] Zou, Q.; Duan, W.; Simmons, D.; Shyo, Y.; Raymond, M. A.; Dorr, R. T.; Hurley, L. H. *J. Am. Chem. Soc.* 2001, 123, 4865.
- [59] Tercel, M.; Stribbling, S. M.; Shephard, H.; Siim, B. G.; Wu, K.; Pullen, S. M.; Bottin, K. J.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.* 2003, 46, 2132.
- [60] (a) Wang, J. J.; Shen, Y. K.; Hu, W. P.; Hsieh, M. C.; Lin, F. L.; Hsu, M. K.; Hsu, M. H. *J. Med. Chem.* 2006, 49, 1442; (b) Hu, W. P.; Liang, J. J.; Kao, C. L.; Chen, Y. C.; Chen, C. Y.; Tsai, F. Y.; Wu, M. J.; Chang, L. S.; Chen, Y. L.; Wang, J. J. *Bioorg. Med. Chem.* 2009, 17, 1172.
- [61] (a) Kamal, A.; Ramesh, G.; Ramulu, P.; Srinivas, O.; Rehana, T.; Sheelu, G. *Bioorg. Med. Chem. Lett.* 2003, 13, 3451; (b) Kamal, A.; Ramesh, G.; Srinivas, O.; Ramulu, P. *Bioorg. Med. Chem. Lett.* 2004, 14, 471; (c). Kamal, A.; Ramulu, P.; Srinivas, O.; Ramesh, G.; Kumar, P. P. *Bioorg. Med. Chem. Lett.* 2004, 14, 4791.
- [62] Kamal, A.; Ramu, R.; Tekumalla, V.; Khanna, G. B. R.; Barkume, M. S.; Juvekar, A. S.; Zingde, S. M.; *Bioorg. Med. Chem.* 2008, 16, 7218.
- [63] Kamal, A.; Reddy, K. S.; Khan, M. N. A.; Shetti, R. V. C. R. N. C.; Ramaiah, M. J.; Pushpavalli, S.N.C.V.L.; Srinivas, C.; Bhadra, M. P.; Chourasia, M.; Sastry, G. N.; Juvekar, A.; Zingde, S.; Barkume M. *Bioorg. Med. Chem.* 2010, 18, 4747.
- [64] Kamal, A.; Kumar P. P.; Sreekanth, K.; Seshadri, B.N.; Ramulu P.; *Bioorg. Med. Chem. Lett.* 2008, 18, 2594.
- [65] Kamal, A.; Reddy, D. R.; Reddy, P. S. M. M.; Rajendar. *Bioorg. Med. Chem. Lett.* 2006, 16, 1160.
- [66] Kamal, A.; Khan, M. N. A.; Reddy, K. S.; Ahmed, S. K.; Kumar, M. S.; Juvekar, A.; Sen, S.; Zingde S.; *Bioorg. Med. Chem. Lett.* 2007, 17, 5345.
- [67] Kamal, A.; Reddy, M. K.; Ramaiah, M. J.; Srikanth, Y. V. V.; Rajender.; Reddy, V. S.; Kumar, G. B.; Pushpavalli, S. N. C. V. L.; Bag, I.; Juvekar A.; Sen, S.; Zingde, S. M.; Bhadra M. P. *ChemMedChem.* 2011, 6, 1665.
- [68] Kamal, A.; Bharathi, E. V.; Ramaiah, M. J.; Dastagiri, D.; Reddy, J. S.; Viswanath, A.; Sultana, F.; Pushpavalli, S. N. C. V. L.; Bhadra M. P.; Srivastava, H. K.; Sastry, G. N.; Juvekar, A.; Sen, S.; Zingde, S. *Bioorg. Med. Chem.* 2010, 18, 526.
- [69] Kamal, A.; Ramu, R.; Tekumalla, V.; Khanna, G. B. R.; Barkume, M. S.; Juvekar, A. S.; Zingde, S. M. *Bioorg. Med. Chem.* 2007, 15, 6868.

- [70] Kamal, A.; Dastagiri, D.; M. Ramaiah, M. J.; Bharathi, E. V.; Reddy, J. S.; Balakishan, G.; Sarma, P.; Pushpavalli, S. N. C. V. L.; Bhadra M. P.; Juvekar, A.; Sen, S.; Zingde, S. *Bioorg. Med. Chem.* 2010, 18, 6666.
- [71] Kamal, A.; Devaiah, V.; Reddy, K. L.; Kumar, M. S. *Bioorg. Med. Chem.* 2005, 13, 2021.
- [72] Kamal, A.; Reddy, J. S.; Ramaiah, M. J.; E. Bharathi, E. V.; Dastagiri, D.; Reddy, M. K.; Pushpavalli, S. N. C. V. L.; Bhadra M. P. *Bioorg. Med. Chem. Lett.* 2010, 20, 5232.
- [73] Kamal, A.; Shankaraiah, N.; Prabhakar, S.; Reddy, C. R.; Markandeya, N.; Reddy, K. L.; Devaiah, V. *Bioorg. Med. Chem. Lett.* 2008, 18, 2434.
- [74] Kamal, A.; Sreekanth, K.; Kumar, P. P.; Shankaraiah, N.; Balakishan, G.; Ramaiah, M. J.; Pushpavalli, S. N. C. V. L.; Ray, P.; Bhadra, M. P. *Eur. J. Med. Chem.* 2010, 45, 2173.
- [75] Puvvada, M. S.; Hartley, J. A.; Jenkins, T. C.; Thurston, D. E. *Nucleic Acids Res.* 1993, 21, 3671.
- [76] Rahman, K. M.; Vassoler, H.; James, C. H.; Thurston, D. E. *ACS Med. Chem. Lett.* 2010, 1, 427.
- [77] Rettig, M.; Langel, W.; Kamal, A.; Weisz, K. *Org. Biomol. Chem.*, 2010, 8, 3179.
- [78] Rettig, M.; Kamal, A.; Ramu, R.; Mikolajczak, J.; Weisz, K. *Bioorg. Med. Chem.* 2009, 17, 919.
- [79] Farmer, J. D.; Rudnicki, S. M.; Suggs, J. W. *Tetrahedron Lett.* 1988, 29, 5105.
- [80] Farmer, J. D.; Gustafson, G. R.; Conti, A.; Zimmt, M.B.; Suggs, J. W. *Nucleic Acids Res.* 1991, 19, 899.
- [81] Reddy, B. S. P.; Damayanthi, Y.; Reddy, B. S. N.; Lown, J. W. *Anti-Cancer Drug Des.* 2000, 15, 225.
- [82] Smellie, M.; Kelland, L. R.; Thurston, D. E.; Souhami, R. L.; Hartley, J. A. *Br. J. Cancer* 1994, 70, 48.
- [83] Thurston, D. E.; Bose, D. S.; Thompson, A. S.; Howard, P. W.; Leoni, A.; Croker, S. J.; Jenkins, T. C.; Neidle, S.; Hartley, J. A.; Hurley, L. H. *J. Org. Chem.* 1996, 61, 8141.
- [84] Hartley, J. A.; Berardini, M. D.; Souhami, R. L. *Anal. Biochem.* 1991, 193, 131.
- [85] Walton, M. I.; Goddard, P.; Kelland, L. R.; Thurston, D. E.; Harrap, K. R. *Cancer Chemother. Pharmacol.* 1996, 38, 431.
- [86] Gregson, S. J.; Howard, P. W.; Hartley, J. A.; Brooks, N. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *J. Med. Chem.* 2001, 44, 737.
- [87] Kamal, A.; Rajender, Reddy, R. R.; Reddy, M. K.; Balakishan, G.; Shaik, T. B.; Chourasia, M.; Sastry, G. N. *Bioorg. Med. Chem.* 2009, 17, 1557.
- [88] Kamal, A.; Prabhakar, S.; Shankaraiah, N.; Reddy, C. R.; Reddy, P. V. *Tetrahedron Lett.* 2008, 49, 3620.
- [89] Suzanne, R.; Thompson, S. *Biochemistry* 2011, 50, 4720.
- [90] Stephen, J.; Philip W. G.; John A. H.; Natalie, H. A.; Lesley, B. J.; Terence, C. A.; Lloyd, R. J.; Thurston, D. E. *J. Med. Chem.* 2001, 44, 737.
- [91] Stephen, J.; Philip W. G.; Darren, H. R.; Hamaguchi, K.; Corcoran, K. E.; Brooks, N. A.; Hartley, J. A.; Jenkins, T. C.; Guille, S. P. M. J.; Thurston, D. E.; *J. Med. Chem.* 2004, 47, 1161.
- [92] Martin, C.; Ellis, T.; Mc Gurk, Claire J.; Jenkins, T. C.; Hartley, J. A.; Waring, M. J.; Thurston, D. E. *Biochemistry*, 2005, 44, 4137.

- [93] Smellie, M.; Bose, D. S.; Thompson, A. S.; Jenkins, T. C.; Hartley, J. A.; Thurston, D. E. *Biochemistry* 2003, 42, 8232.
- [94] Alley, M. C.; Hollingshead, M. G.; Cox, P. C. M.; Waud, W. R.; Hartley, J. A.; Howard, P. W.; Gregson, S. J.; Thurston, D. E.; Sausville, E. A. *Cancer Res.* 2004, 64, 6700.
- [95] Kamal, A.; Shankaraiah, N.; Reddy, C. R.; Prabhakar, S.; Markandeya, N.; Srivastava, H. K.; Sastry, G. N. *Tetrahedron*. 2010, 66. 5498.

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Over the recent years, medicinal chemistry has become responsible for explaining interactions of chemical molecules processes such that many scientists in the life sciences from agronomy to medicine are engaged in medicinal research. This book contains an overview focusing on the research area of enzyme inhibitors, molecular aspects of drug metabolism, organic synthesis, prodrug synthesis, in silico studies and chemical compounds used in relevant approaches. The book deals with basic issues and some of the recent developments in medicinal chemistry and drug design. Particular emphasis is devoted to both theoretical and experimental aspect of modern drug design. The primary target audience for the book includes students, researchers, biologists, chemists, chemical engineers and professionals who are interested in associated areas. The textbook is written by international scientists with expertise in chemistry, protein biochemistry, enzymology, molecular biology and genetics many of which are active in biochemical and biomedical research. We hope that the textbook will enhance the knowledge of scientists in the complexities of some medicinal approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of medicinal chemistry and drug design.

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