Recent Developments in Novel Pyrrolo[2,1-*c*][1,4]Benzodiazepine Conjugates: Synthesis and Biological Evaluation

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Abstract: The biological activity of many low molecular weight antitumor compounds appear to be related to their mode and specificity of interaction with particular DNA sequences. Such small molecules are of considerable interest in chemistry, biology and medicine. Most of the anticancer drugs employed clinically exert their antitumor effect by inhibiting nucleic acid (DNA or RNA) or protein synthesis. Inhibition can occur for example through cross-linking of bases in DNA or binding to and inactivation of enzymes necessary for the synthetic processes. It is evident that DNA is an important cellular target for many anticancer agents. Much information has been obtained from molecular genetics, i.e. replication of DNA and its transcription to RNA, which provides the template for protein synthesis. DNA is a well-characterized intracellular target but its large size and sequential nature makes it an elusive target for selective drug action. Binding of low molecular weight ligands to DNA causes a wide variety of potential biological responses. In this context PBDs (pyrrolo[2,1-*c*][1,4]benzodiazepines), a group of potent naturally occurring antitumor antibiotics produced by various *Streptomyces* species, are one of the most promising types of lead compounds. They differ in the number, type and position of substituent in both their aromatic A-ring and Py C-rings, and in the degree of saturation of the C-rings which can be either fully saturated or unsaturated at either C2-C3 (endocyclic) or C2 (exocyclic). There is either an imine or carbinolamine methyl ether at the N10-C11 position. This latter is an electrophilic center responsible for alkylating DNA. In the search for compounds with better antitumor selectivity and DNA sequence specificity many PBD analogues have been synthesized in an attempt to increase their potency against tumor cells. We review here recent progress on pyrrolo[2,1-*c*][1,4]benzodiazepine (PBDs) analogues and their conjugates, also the progress and developments of PBD conjugates with polyamides (information reading molecules in the minor groove of DNA). For example, the cross-linking efficiency of PBD dimers is much greater than that of other cross linkers including cisplatin and melphalan. A large number of PBD dimers and polyamide conjugates with varying linker lengths and bearing different heterocycles at different positions in the PBD ring synthesized in our group and their pharmacological properties have been reviewed.

INTRODUCTION

The pyrrolo[2,1-*c*][1.4]benzodiazepine (PBD) antitumor antibiotics are produced by various strains of *Streptomyces* and the members of this family include anthramycin **1** [1], mazethramycin **2** [2], prothramycin **3** [3], tomaymycin **4** [4], prothracarcin **5** [5], sibanomycin **6** [6], neothramycin A **7** and B **8** [7], DC-81 **9** [8,9], sibiromycin **10** [10], chicamycin **11** [11] and abbeymycin **12** [12].

They differ in the number, type and position of substituents in both their aromatic A-ring and pyrrole C-rings, and in the degree of saturation of the C-ring which can either be fully saturated or unsaturated at either C2-C3 (endocyclic) or C2 (exocyclic). There is either an imine or carbinolamine methyl ether at the N10-C11 position, which is an electrophilic center responsible for alkylating DNA. Most of the known natural PBDs have (S) configuration at the chiral C11a position, which provides them with a right handed twist when viewed from the C-ring towards the A-ring. This gives them the appropriate three-dimensional shape for isohelicity with the minor groove of B-DNA leading to a snug fit at the binding site. Racemization at C11a can significantly reduce both DNA binding activity and *in vitro* cytotoxicity [13].

The N10-C11 imine moiety may exist in the hydrated carbinolamine or carbinolamine methyl ether form depending

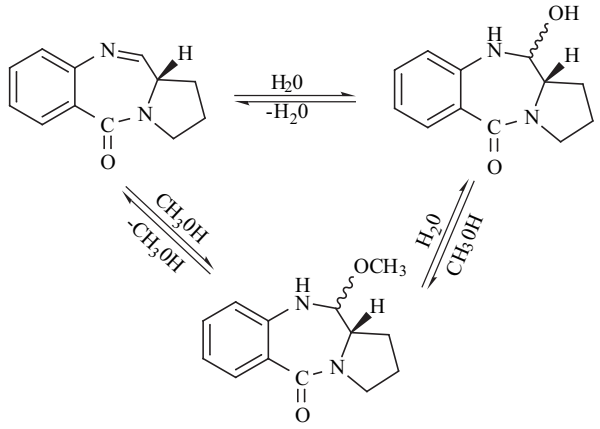

Fig. (1). Carbinolamine - methyl ether - imine interconversions in PBD.

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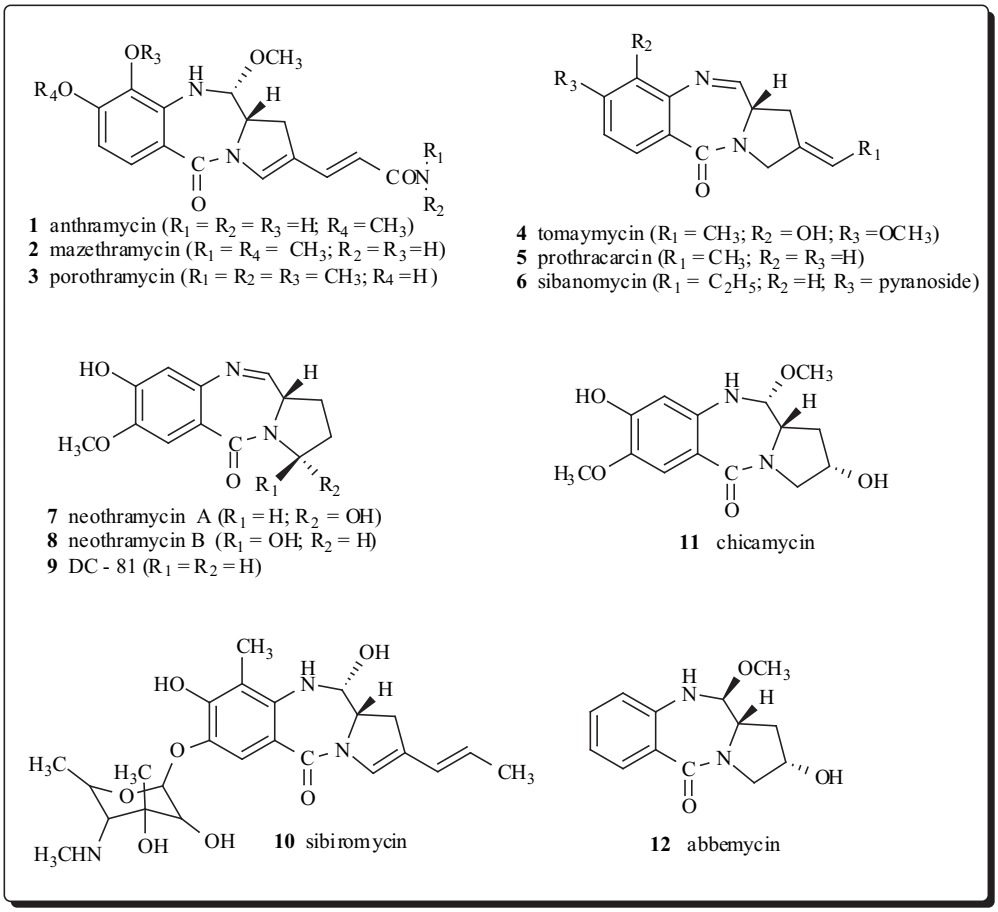


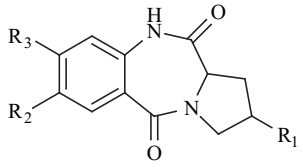
upon the method of isolation or synthetic work up. Imine and methyl ether forms may be interconverted by dissolution of imine in methanol or by several cycles of refluxing the methyl ether in chloroform followed by evaporation of the solvent in vacuo (Fig. **1**) [14,15].

PBD Analogues

As mentioned above there is considerable interest in developing low molecular weight molecules with sequence selectivity DNA interactive properties as tools for molecular biology and as possible therapeutic agents to inactivate particular genes. Therefore various PBD analogs have been synthesized for the above purposes [16-18]. A series of PBD-5, 11-diones **13-27** has been synthesized and evaluated for DNA binding by thermal denaturation and fluoresence quenching studies with calf thymus (CT) DNA [19]. The results indicated that two compounds of the series **13** and **14**, elevate the melting point of DNA by 2.9 ± 0.6 and 3.3 ± 0.8 K, respectively (Table 1). Similarly a significant quenching of the fluorescence of the dihydroxy analog **14** was observed upon interaction with CT-DNA. As control the dihydroxy isomer **15** with the reverse stereochemistry at C2 and the non-substituted dialactam **16** failed to increase the DNA melting point or exhibit significant quenching upon interaction with DNA. Removal of C2-OH group (dilactam **27**), while retaining the C8-OH and C7-OCH_3 groups leads to a loss of significant binding, indicating that the C2-OH substituents play an important role in the binding process. Similarly removal of the C8-OH group and C7-OCH_3 substituents, while retaining the C2-OH in either R **19** or S **20** configuration leads to loss of binding, indicating the importance of A-ring substituents in the binding process. Substitution of the C8-hydroxyl group with a benzyl substituent (dilactam **16**) leads to loss of binding, suggesting that either the C8-OH proton is involved in hydrogen bonding or that the benzyl group causes an undesirable steric interaction. Preliminary experiments with GC- and AT-rich polymers suggest some sequence dependent properties for dilactams **13** and **14**. Overall these results indicate a highly specific structural requirement for DNA binding. Molecular modelling with d (GTAGATC), d (GCAGATC) and d (GCGTAGC) duplex sequences has provided a model, based on hydrogen bonding between the dihydroxy dilactams **14** and DNA, that rationalizes some of the results obtained. It is possible that the observed interactions represent the non-covalent (binding) component of the interaction covalently binding anthramycin type antitumor antibiotics with DNA.

Gillard *et al.* [20] have synthesized a series of thiazolobenzodiazepines **28-42** and pyrrolobenzodiazepines **43-47.** In the thiazole series the triazolic compound **41** exhibited good affinity for the benzodiazepine receptor and correlated with anti-convulsant activity and low toxicity in mice [21]. Improvement of benzodiazepine receptor affinity could be realized by substitution of the triazole ring by ester substitutions as in the case of flumazenil **48.**

Table 1. Change in Melting Temperature (ΔT_m) of CT-DNA After Interaction with Dilactams 13-27



Dilactam	R_1	R_2	R_3	C2[a]	T_m (K)[b]
13	$OCOCH_3$	OCH_3	CH_3COO	R	2.9 ± 0.6
14	OH	OCH_3	OH	R	3.3 ± 0.8
15	OH	OCH_3	OH	S	<0.5
16	OH	OCH_3	OBn	R	<0.5
17	OH	OCH_3	OBn	S	<0.5
18	H	H	H	–	<0.5
19	OH	H	H	R	<0.5
20	OH	H	H	S	<0.5
21	$OCONH_2$	H	H	R	<0.5
22	Cl(C2=C3)	H	H	–	<0.5
23	$OCOCH_2CH_2N$(morph)[c]	H	H	R	<0.5
24	$OCOCH_2CH_2Cl$[d]	OCH_3	$OCOCH_2CH_2C$	R	<0.5
25	$OCONH_2$	OCH_3	$OCONH_2$	R	<0.5
26	$OCOCH_2CH_2Cl$[d]	H	H	R	<0.5
27	H	OCH_3	OH	–	<0.5

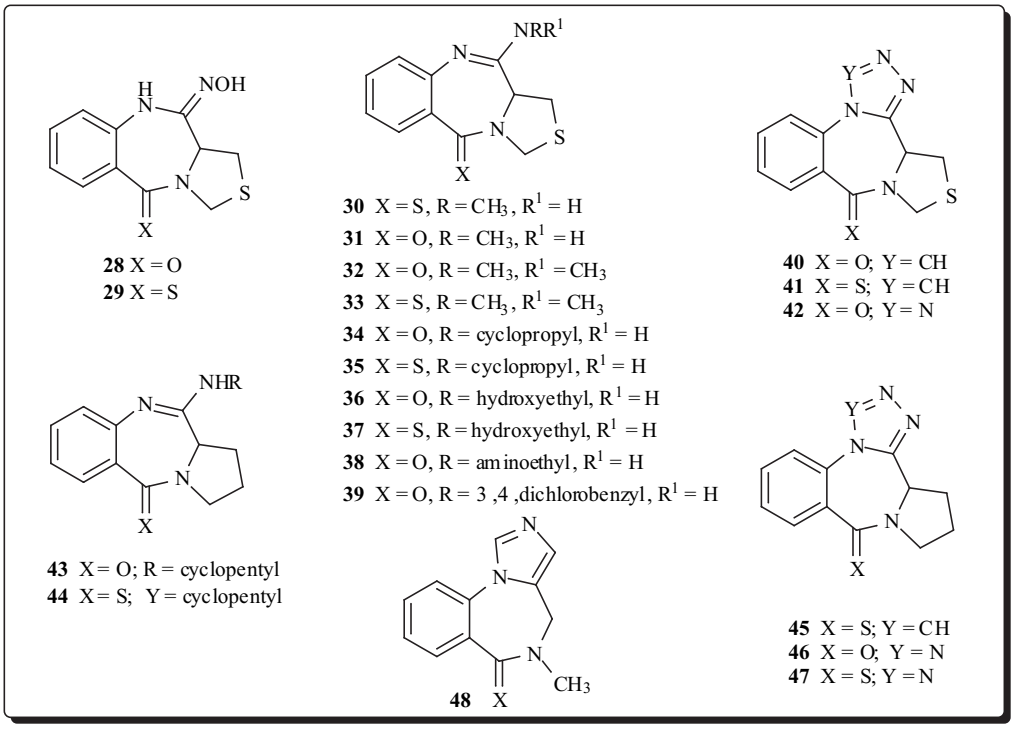
[a]Stereochemistry at C2 atom.

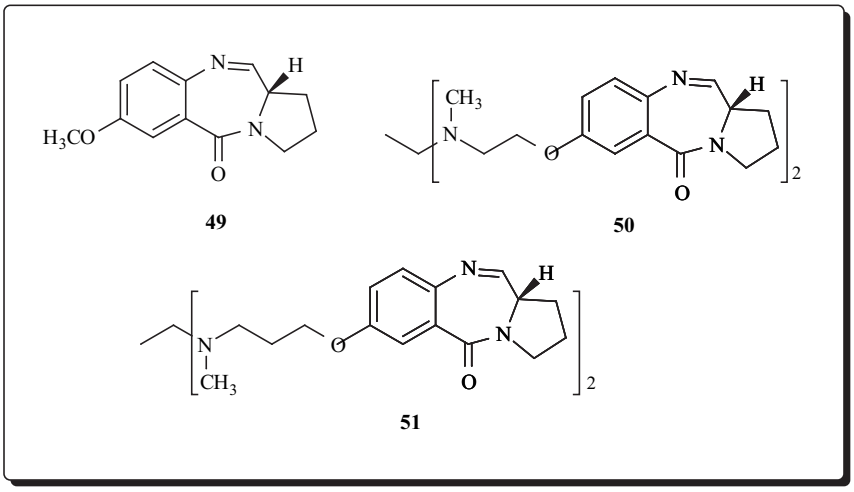
[b]Change in melting temperature relative to CT-DNA.

[c]–N (morph) refers to morpholin-1-yl.

[d]Incubated at 310K for 2 h prior to measurement.

The DNA binding properties of the anthramycin analogs **49-51** have been investigated by fluorescence spectroscopy [22]. A substantial fluorescence enhancement occurs when PBDs are covalently attached to duplex DNA which to



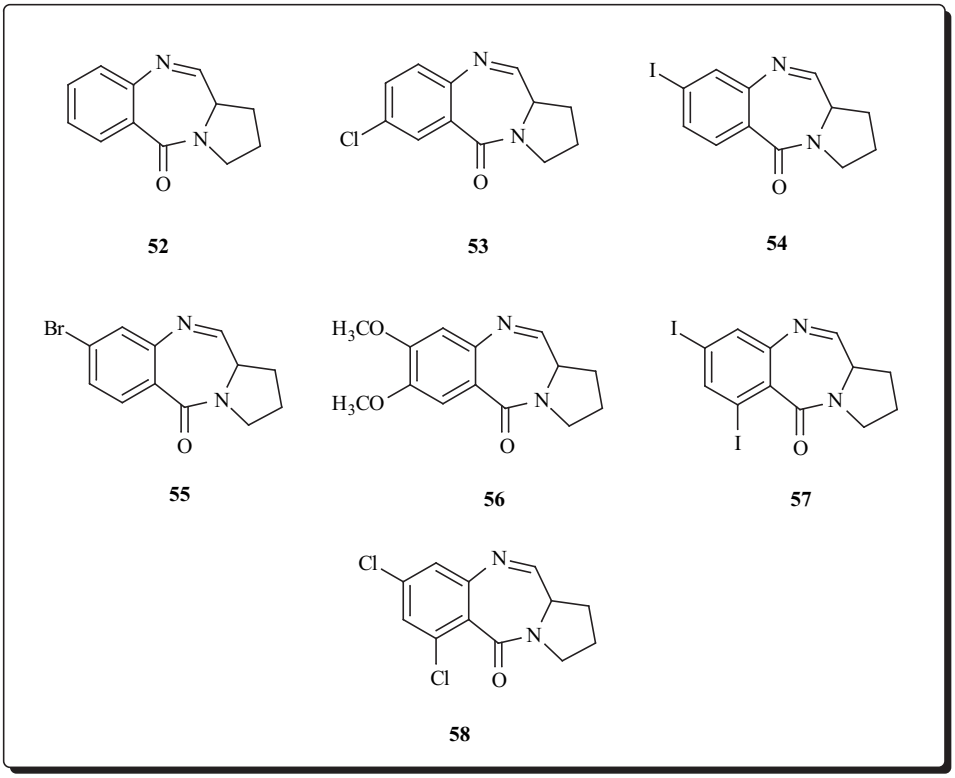


49 **50** **51**

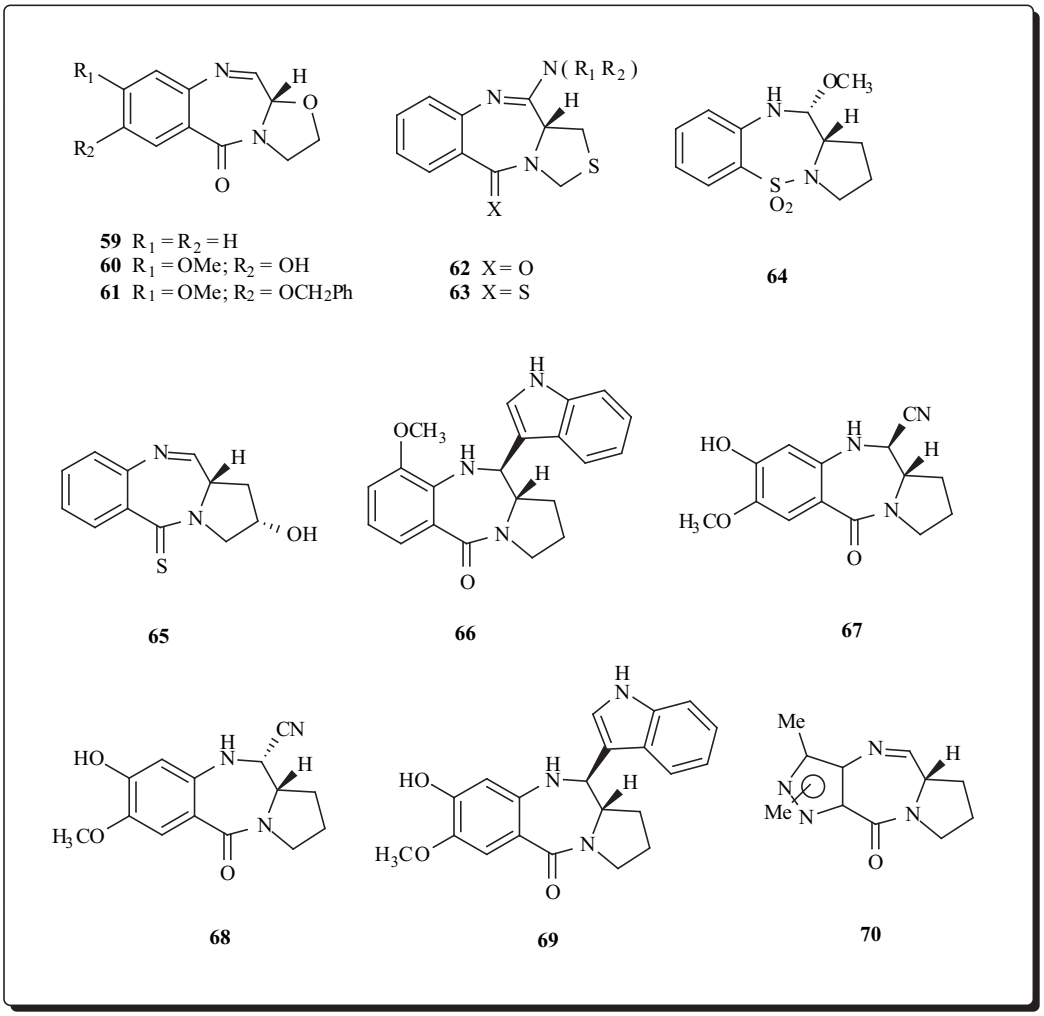
shows that the presence of RNA, or single stranded DNA, or protein had any effect on the degree of fluorescence enhancement resulting from the incubation of **50** and **51** with DNA. The enhancement was found to be dependent on the presence of the imine functionality in each of the compounds. A wavelength of 320 nm was used to excite the chromophore and its emission wavelength maximum was 420 nm. The PBD ring system exhibits exceptionally favorable fluorescence polarization anisotropy (FPA) decay characteristics. For these studies a simpler analog **49** was used and the time-resolved maximum FPA for **49** in glycerol at 25ºC is 0.28. These results indicate that the PBD family of an antibiotic could serve as sensitive probes of DNA dynamics in the 0.1 to 3.5 ns time range.

O' Niel *et al.* [23] have synthesized a number of anthranilic acid derivatives, which possess a substituent in the aromatic, A ring **52-58**. They have used Dess-Martin periodinane reagents in the direct preparation of the PBD ring system from an amino alcohol precursor. This eliminates the need to prepare the azido alcohol and to carry out the Staudinger/aza-Wittig-cyclization, considerably shortening the synthetic sequence. Selected novel PBDs compound **53-55** were screened for *in vitro* cytotoxicity against two human A2780 ovarian carcinoma cell lines and they showed activity in the order 7-bromo > 7-Iodo> 8-chloro with the bromo derivative having significant cytotoxic potency. These three PBD monomers retain partial or complete activity towards cisplatin resistant A2780 *cis r* where the cell line has acquired 11-fold resistance towards this clinical antitumor agents.

A number of additional PBD analogs has been synthesized, oxazolo [2,3-c][1,4] benzodiazepines **59-61**



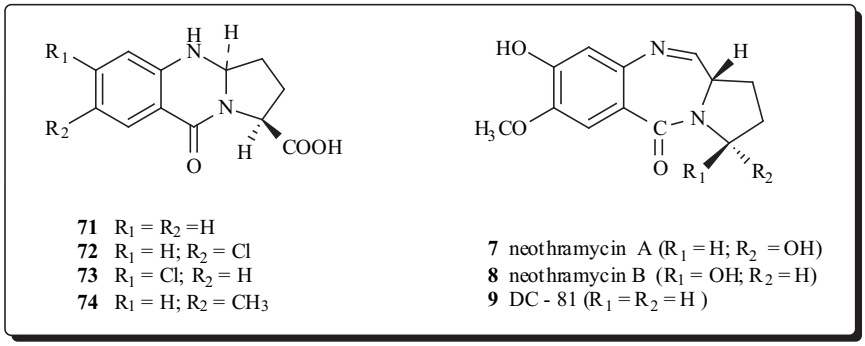
52 **53** **54** **55** **56** **57** **58**

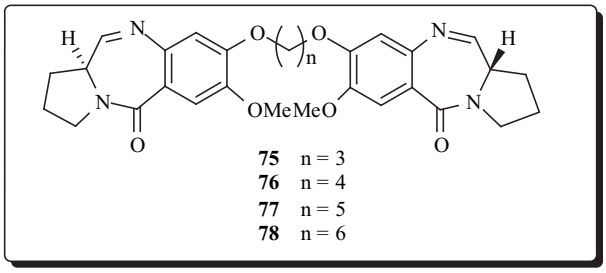


[24], 1,2,3,11a-tetrahydro-11-alkylamino-5H-thiazolo[4.3-c][1,4]benzodiazepine-5-one and 5-thione **62, 63** [25], sulfone anlogs of abbemycin **64** [26], 5-thioabbyemycin **65** [27], tilivalline **66-69** [28,29], pyrazolo [4,3-e] pyrrolo [1,2a] [1,4] diazepine **70** [30].

Fovehat *et al.* [31] have reported the synthesis of new 2,3,4,4a-tetrahydro pyrrolo [2,1-*c*][1,4] quinazolin-9 (1H)-1-carboxylic acids **71-74** from 1,10,11,11a-tetrahydropyrrolo-[2,1-*c*][1,4] benzodiazepine-5, 11-diones in good yield. Rearrangement of pyrrolo[2,1-*c*][1,4] benzodiazepines into cyclopenta [b][1,4] benzodiazepines has been reported under microwave or conventional heating conditions [32]. Prabhu *et al.* [33] used tetrathiomolybdate mediated reductive cyclization of ω azido carbonyl compounds for the efficient synthesis of pyrrolo [2,1-*c*][1,4] benzodiazepines and was applied successfully for the synthesis of DC-81 **7-9** and benzoyl DC-81. Kamal *et al.* have used hexamethyldisilathiane (HMDST) [34] N, N-dimethylhydrazine and $FeCl_3.6H_2O$ [35], bakers yeast [36], SmI_2 [37], and TMSI [38] for reductive cyclization.

Bose and Thurston *et al.* [39-41] synthesized C8-linked PBD dimers **75-78** and reported some of their biological activity but the detailed biological studies for most of the analogs have not been discussed. The C8-linked PBD dimers **75-78** form an irreversible interstrand cross-link between two guanine bases within the minor groove of DNA. According to molecular modelling and NMR studies, the DSB 120, **75** spans six base pairs actively recognizing a





75 n = 3
76 n = 4
77 n = 5
78 n = 6

central 5'-GATC sequence. DNA binding of **75** was observed through thermal denaturation studies with calf thymus DNA. (ΔT_m > 15.1 ^{0}C for a 5:1ratio of DNA: ligand at 37 ^{0}C for 18H.) Crosslinking efficiency was investigated by using agarose gel electrophoresis assay. The results indicate that **75** is a remarkably efficient cross-linking agent. After 2h at 37˚C, crosslinking is measurable down to 0.01 nM (drug: nucleotide ratio=1.0). It is 50 fold more effective than major groove cross linker's mechlorethamine and cisplatin, and 300 times more efficient than melphalan and similar in efficiency to the rigid CC-1065 dimer U-77, 779. Molecular model studies of **75** with d $(CGYGXXCYCG)_2$ suggested that spatial separation of the PBD units is optional for spanning six base pairs with preferences for 5'-PuGATCPy or 5'-PuGATCPu sequence, and that it actively recognizes the d $(GATC)_2$ sequence (Fig**.2**).

PBD – Polyamide Conjugates

Baraldi *et al.* [42] synthesized a PBD-polyamide conjugate **79** which is a combination of the naturally occuring distamycin and the PBD related to the naturally occuring anthramycin. *In vitro* studies on cell growth and arrested PCR clearly demonstrated that the hybrid is much more active than distamycin and PBD in inhibiting cell

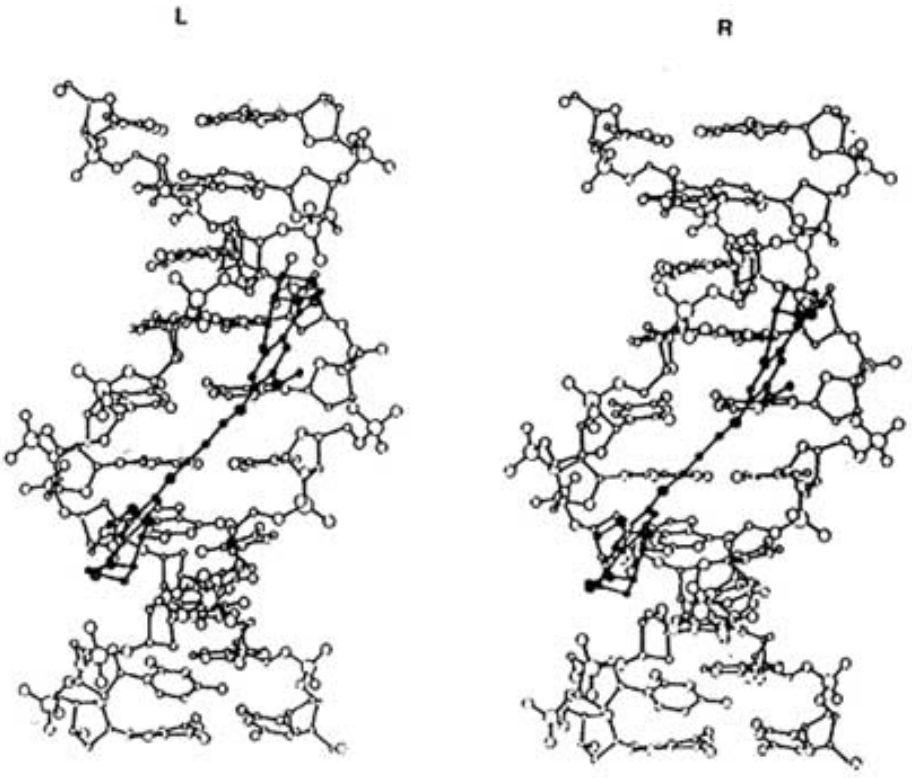
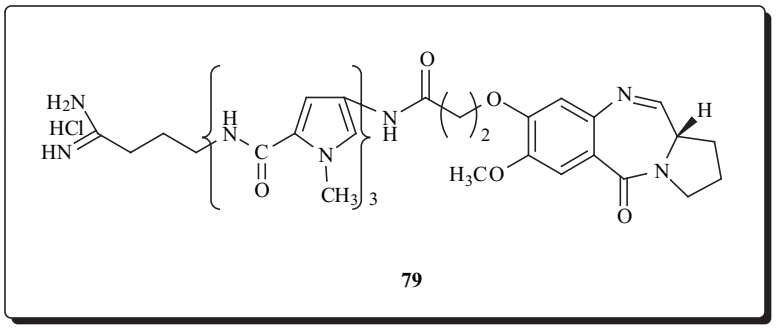


Fig. (2). Stereoview of the cross-linked d $(CICGATCICG)_2$-PBD dimmer adduct following X-PLOR refinement. The dimmer 268 (shown in bold) is located in the minor groove of B-DNA, spanning bases C3-18, and is bound covalently to G4 bases on adjacent strands. All H-atoms have been removed for clarity.

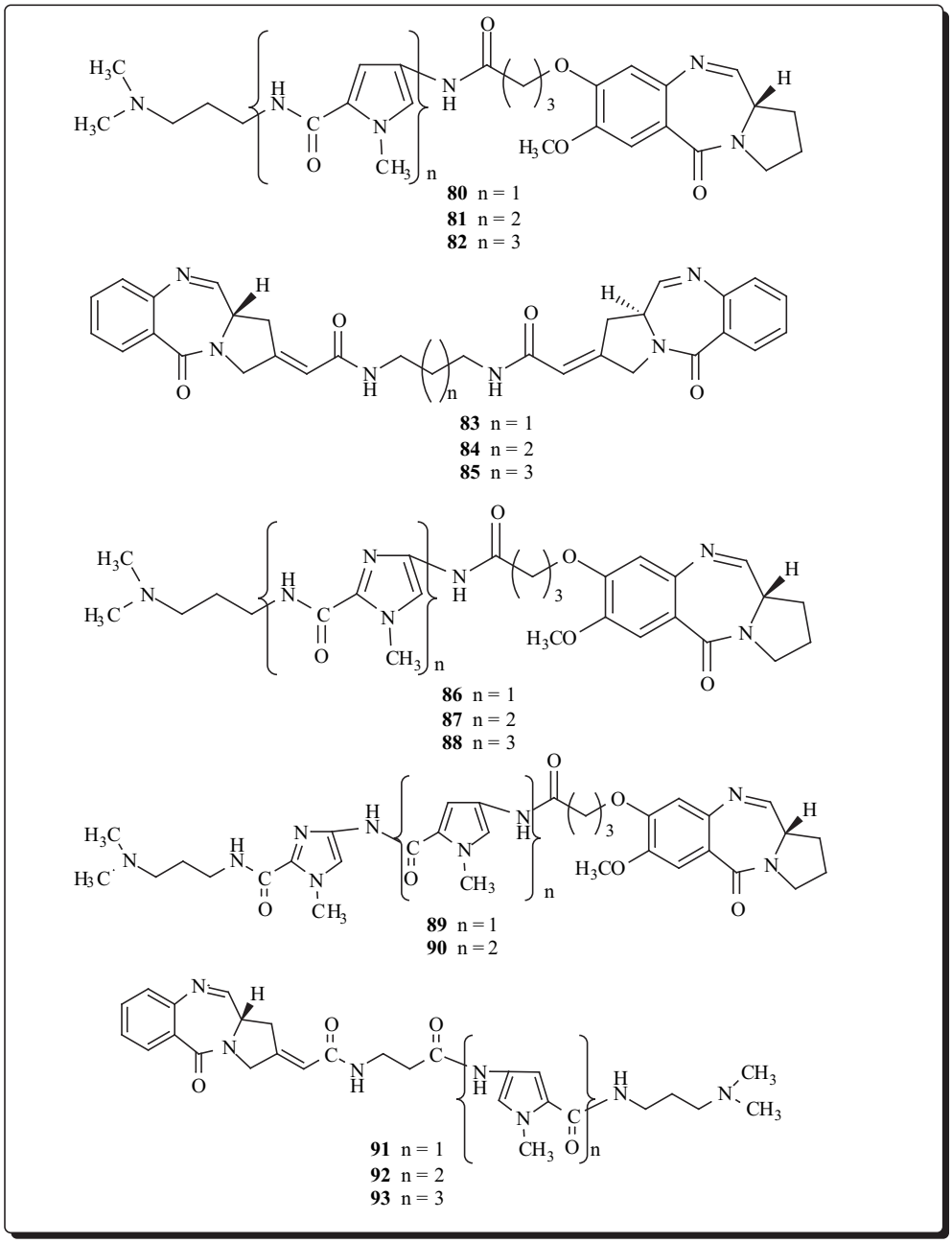


79

proliferation of neoplastic cell lines and binding to DNA sequences. The DNA binding selectivity of compound **79** is different from that of distamycin, as it is capable to bind to G+C rich elements.

A number of 2,2'-PBD dimers and PBD- polyamide conjugates **80-93** have been synthesized by Lown *et al.* [43-47] bonded through the C-8 and C-2 position with a suitable linker. The conjugates were synthesized by an amidic linkage by coupling the amine of the polyamide unit with the acid moiety of the linker attached to the PBD system. The PBD-polyamide conjugates **81-82** and 2,2'-PBD dimmers **83-85** were evaluated for the cytotoxic activities [45] against 9 panels containing 60 human cell lines. A 48-hour continuous drug exposure protocol was used, and a sulfurhodamine B protein assay was used to estimate cell viability or growth.

According to the data from **Table-2** compounds **81**, **82** and **85** have cytotoxic potency against many cell lines. Compound **82** exhibits a wide spectrum of activity against 17 cell lines in 6 cell panels with LC_{50} values less than 9.0 μM both in the test 1 and in test 2. The average LC_{50} values of compound **82** against colon cancer COLO-205, HCT-116 and HCT-15 cell lines are 6.0, 7.0, 8.0 μM respectively. In the CNS cancer panel, the growth of U251 cell line was affected by compound **82** with a LC_{50} value as 5.5 μM. Most of the cell lines in the melanoma panel were affected by **82** at low concentrations (5.0 to 8.0 μM). In the ovarian cancer panel the growth of OVCAR-8 cell line was affected by compound **82** with the LC_{50} value as 5.0 μM. Almost all of 6 cell lines in the renal cancer panel were affected by compound **82** which also exhibits high cytotoxic potency against ACHN, CAKI-1, RXF 393, SN 12C, TK 10 and UO 31 cell lines with the LC_{50} values of 5.0, 3.5, 5.0, 5.0,



6.0 and 5.0 μM respectively. Compound **82** exhibits a selective cytotoxic potency in the breast cancer panel in which MCF 7, MDA-MB-435 and MDA-N cell lines were affected with LC_{50} values less than 8.0 μM. The cell growth of NCI/ADR-RES, HS 587 T and T-47D cell lines were not affected by compound **82** (LC_{50} > 100 μM). In this study compound **82** exhibits low cytotoxic potency against all of the leukemia cell lines (LC_{50} > 100 μM).

Table-2. ***In vitro*** **Cytotoxic Potency (LC_{50}s) of Pyrrolo[2,1-*c*][1,4]Benzodiazepine (PBD)-Polyamide Conjugates and 2, 2'-PBD Dimers Againest 9 Panels of Human Cell Lines**

LC_{50}s (μM)

Panels/Cancer cell line	81			82			83			84			85		
	Test 1	Test 2	Average[b]	Test 1	Test 2	Average	Test 1	Test 2	Average	Test 1	Test 2	Average	Test 1	Test 2	Average
Leukemia															
CCRF-CEM0(TB)	-	>[a]	-	-	>	-	-	-	-	-	>	-	>	>	-
HL-60(TB)	38	>	38	7.6	>	7.6	>	-	-		79	79	69	>	69
K-562T-4	-	>	-	-	>	-	>	-	-	74	>	74			
MOLT-4		>-	-	-	>	-	>	-	-	9.2	>	9.2	>		>
RPMI-8226		>>	-	>	>	-	>		--	>	>	-	>	>	-
SR	>	>	-	5.4	>	5.4	13	-	13	7.6	>	7.6	>	>	-
Non-small cell lung cancer															
A549/ATCC	49	>	49	93	>	93	>	-	-	>	>	-	>	>	-
HOP-62	46	45	45.5	98	6.4	52.2	77	-	77	>	74	74	69	73	71
HOP-92	56	52	54	6.0	-	6.0	63	-	63	45	-	45	45	-	45
NCT-H226	34	>	34	85	-	85	63	-	63	73	>	73	92	>	92
NCI-H23	33	44	38.2	5.6	33	19.3	59	-	59	49	69	59	66	63	64.5
NCI-H322M	92	81	86.5	59	51	55	57	-	57	57	58	57.5	59	53	56
NCI-H460	7.5	>	7.5	50	95	72.5	70	-	70	74	84	79	75	70	72.5
NCI-H522	-	>	-	0.9	-	0.9	9.0	-	9.0	72	>	72	8.2	6.4	7.3
EKVX	7.1	-	71	54	-	54	73	-	73	68	-	68	82	-	82
Colon cancer															
COLO 205	55	67	61	5.7	6.6	6.15	53	-	53	39	51	45	8.9	>	8.9
HCC-2998	39	>	39	55	56	55.5	60	-	60	57	62	59.5	59	56	57.5
HCT-116	45	45	45	5.4	9.1	7.25	58	-	58	55	53	54	49	54	51.5
HCT-15	54	66	60	7.6	8.8	8.2	75	-	75	64	96	80	67	41	54
HT 29	91	68	79.5	6.6	18	12.3	63	-	63	56	86	71	58	71	64.5
KM12SW-620	95	53	74	>	55	55	60	-	60	69	57	63	>	59	59
SW-620	57	>	57	7.4	>	7.4	>	-	-	>	-	-	>	>	-
CNS cancer															
SF-268	42	93	67.5	6.2	7.2	6.7	56	-	56	55	64	59.5	57	43	50
SF-295	>	>	-	64	50	57	71	-	71	70	58	64	60	58	59
SF-539	49	54	51.5	8.2	46	27.1	66	-	66	64	60	62	58	>	58
SNB-19	46	70	58	33	65	32.5	60	-	60	60	61	60.5	54	66	60
SNB-75	59	19	39	58	16	37	59	-	59	71	54	62.5	66	52	59
U251	17	22	19.5	5.3	5.9	5.6	51	-	51	33	39	36	29	38	33.5

(Table 2). contd.....

Panels/Cancer cell line	81			82			83			84			85		
	Test 1	Test 2	Average[b]	Test 1	Test 2	Average	Test 1	Test 2	Average	Test 1	Test 2	Average	Test 1	Test 2	Average
Melanoma															
LOX IMVI	35	32	33.5	4.9	5.2	5.15	65	-	65	39	8.9	23.95	21	6.7	13.85
MALME-3M	48	55	51.5	5.6	7.0	6.3	56	-	56	37	59	48	30	27	28.5
M14	37	48	42.5	5.5	6.1	58	54	-	54	48	45	46.5	37	38	37.5
SK-MEL-2	56	38	47	25	27	26	58	-	58	55	56	55.5	55	58	56.5
SK-MEL-28	44	64	55	17	8.5	12.7	66	-	66	62	78	70	57	67	62
SK-MEL-5	7.7	8.1	7.9	36	7.6	21.8	56	-	56	54	60	57	53	>	53
UACC-257	44	53	48.5	8.3	8.6	8.45	55	-	55	46	>	46	42	46	44
UACC-62	33	8.4	20.7	24	24	24	54	-	54	55	54	54.5	55	57	56
Ovarian cancer															
IGROV1	49	60	54.5	33	22	27.5	55	-	55	50	56	53	47	50	48.5
OVCAR-3	45	47	46	-	6.7	6.7	81	-	81	>	63	63	68	48	58
OVCAR-4	75	43	59	>	6.0	6.0	69	-	69	>	45	45	>	39	39
OVCAR-5	61	57	59	21	39	30	61	-	61	59	64	61.5	62	58	60
OVCAR-8	51	47	49	5.5	5.9	5.7	59	-	59	48	7.6	27.8	40	5.8	22.9
SK-OV-3	49	43	46	59	51	55	71	-	71	75	55	65	58	54	56
Renal cancer															
786-0	30	36	33	4.7	5.2	4.95	37	-	37	6.2	6.4	6.3	6.5	6.1	6.3
A498	-	0.2	0.2	52	53	52.5	56	-	56	57	58	57.5	57	60	58.5
ACHN	36	27	31.5	5.4	5.6	5.5	53	-	53	33	39	36	8.6	6.7	7.65
CAKI-1	54	4.4	29.2	6.5	1.3	3.9	55	-	55	36	6.0	21	72	6.1	39.05
RXF 393	23	8.9	15.95	6.1	5.7	5.9	57	-	57	48	43	45.5	42	31	36.5
SN 12C	53	44	48.5	4.7	6.2	5.45	79	-	79	21	52	36.5	8.5	65	36.75
TK-10	49	48	48.5	6.9	6.0	6.45	58	-	58	53	47	50	44	15	29.5
UO-31	44	45	44.5	5.4	5.8	5.6	55	-	55	21	17	19	7.1	7.7	7.4
Prostate cancer															
PC-3	49	54	51.5	31	6.9	18.95	56	-	56	52	54	53	52	53	52.5
DU-145	56	37	46.5	47	19	33	69	-	69	62	53	57.5	57	53	55
Breast cancer															
MCF7	74	43	58.4	>	7.7	7.7	>	-	-	>	63	63	>	76	76
NCI/ADR-RES	73	96	84.5	>	>	-	>	-	-	95	>	95	>	>	-
MDA-MB-231/ATCC	25	62	43.5	27	7.2	17.1	54	-	54	0.06	55	27.53	52	62	57
HS 578T	>	>	-	-	>	-	>	-	-	>	>	-	>	>	-
MDA-MB-435	90	46	68	7.5	6.0	6.75	61	-	61	59	43	51	>	35	35
MDA-N	50	49	54.5	9.3	6.6	7.95	60	-	60	55	47	51	62	53	57.5
BT-549	49	50	54.9	6.7	28	17.35	56	-	56	51	54	52.5	51	52	51.5
T-47D	>	>	-	>	>	-	>	-	-	>	>	-	>	>	-

[a]LC_{50} > 100μM. [b]Average value of LC_{50} in test 1 and test 2

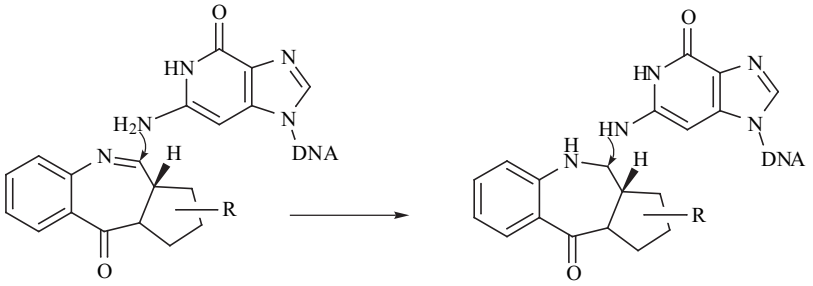


Fig. (3). PBD - DNA adduct.

Compound **81** exhibits marked cytotoxic potency against renal cancer cell lines A 498 and CAKI-1, with the LC_{50} values of 0.2 and 4.0 μM respectively, and it also exhibits cytotoxicity against melanoma SK-MEL-5 cell line, with the LC_{50} value is less than 8.0 μM. Compound **84** shows high potency towards the breast cancer cell line MDA-MB-231/ATCC with the LC_{50} value 0.06 μM. Compound **85** exhibits promissing cytotoxic potency against non-small cell lung cancer NCI-H522 cell line and renal cancer 786-0, ACHN, and UO-31 cell lines with the LC_{50} values less than 8.0 μM. The cytotoxicities of compounds **83** and **84** were relatively less than those of the compounds **81**, **82** and **85**. None of the leukemia cancer cell lines were affected by compounds **81**-**85**.

Compound **82** which bears three pyrrole rings has the highest cytotoxic potency when compared with compound **81** which bears only 2 pyrrole rings. All of the 2,2' PBD dimmers (**83**, **84** and **85**) have relatively lower cytoxicty when compared with compound **82**. This study found that 2, 2' PBD dimers and PBD-polyamide conjugates are actively cytotoxic against many human cancer cell lines. Compound **82** has a wide spectrum of anticancer activity, which affects the cell growth of 17 cell lines in six cancer panels with LC_{50} values lower than 9.0 μM. These cell lines include colon cancer, COLO 205, HCT-116 and HCT-15 cell lines, melanoma cancer LOX IMVI, MALME-3M, M14 and UACC-257 cell lines, ovarian cancer OVCAR-8 cell line, renal cancer, ACHN, CAKI-1 RXF 393, SN 12C, TK-10 and UO-31 cell lines, breast cancer MDA-MB-435 and MDA-N cell lines. Compounds **83** and **84** exhibits lower cytotoxicity compared with compounds **81**, **82** and **85**. Compound **84** exhibited significant cytotoxicity in breast cancer cell line MDA-MB-231/ATCC with the LC_{50} value 0.06 μM. The biological study of compounds **86-93** is under evaluation and their results will be disclosed in due course.

Drug-DNA Interactions

The mechanism of action of the PBDs is associated with their ability to form an adduct in the minor groove of DNA, 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 (Fig**. 3**). X-ray diffraction studies on crystals of anthramycin methyl ether show that the molecule is twisted 40-50º from one end to the other along the long axis, and this might fit into one of the groove of DNA. In the CPK models, the drug fits snugly within the narrow groove without distortion of the DNA helix; the bulky amino sugar of sibiromycin is the only part of any of the antibiotic which extends outside the groove of DNA [48,49]. In the sibiromycin, the amino sugar may further stabilize the adduct by the interaction with deoxyribose phosphate backbone of DNA [50].

Hurley *et al.* Have considered three different mechanisms for the reaction of PBD antibiotics with DNA [51] (Fig**. 4**).

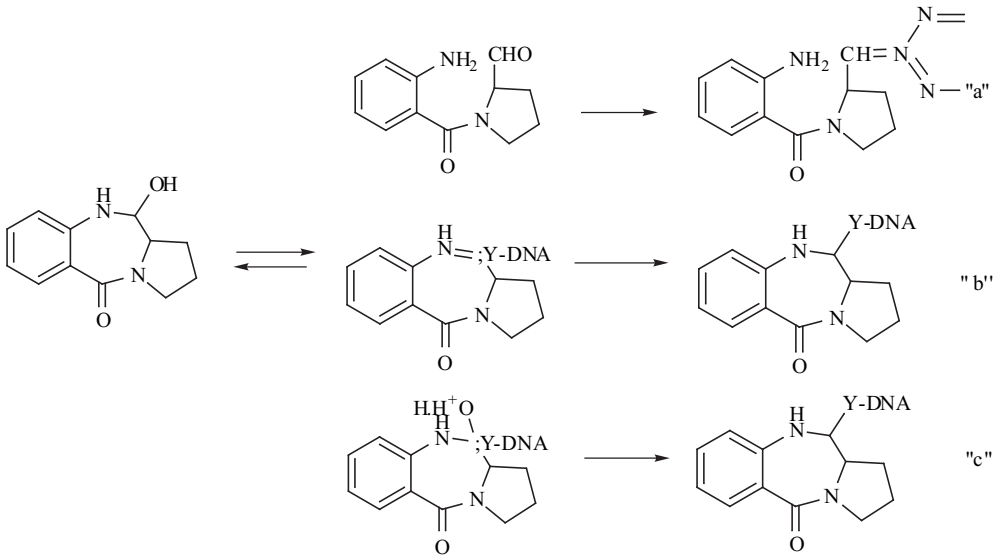
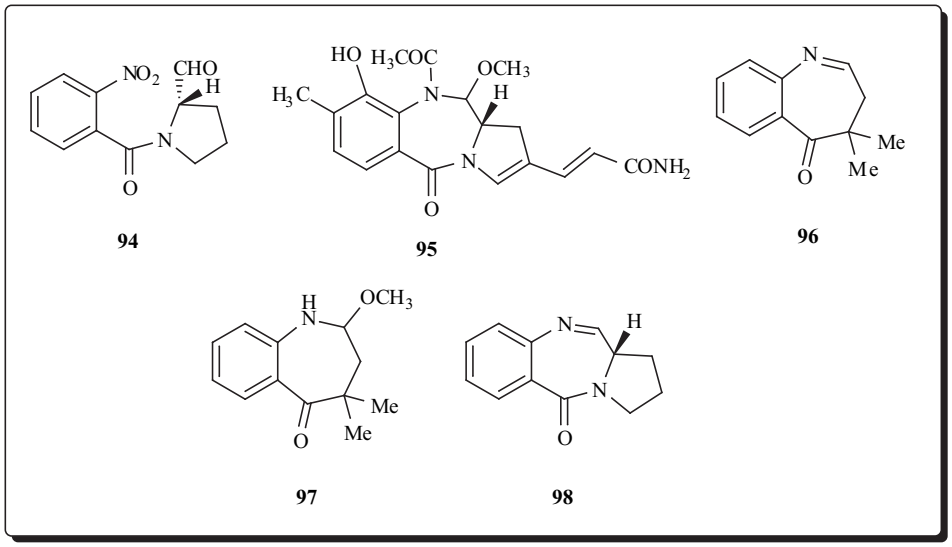


Fig. (4). Proposed mechanism for the PBD - DNA complex.



94 **95** **96**

97 **98**

The mechanism 'c' is considerd favorable by them based on the fact that (i) reduction of DNA antibiotic complexes did not result in any increase in the amount of acid stable complex, which rules out mechanism 'a' and (ii) the imine of sibiromycin (anhydrosibiromycin) is biologically unreactive [52]. Further evidence against mechanism 'a' is that the nitroaldehyde **94** failed to react with DNA. Studies by Lown *et al.* are not in agreement with this mechanism, since the anthramycin derivative **95**, which has a free hydroxyl group at the C9 position but has an acetyl group on the nitrogen instead of hydrogen for anthramycin did not react with DNA. If the proposed SNC 'a' mechanism is operative, compound **95** should also react with DNA. The authors consider that mechanism 'b' is operative, since the azomethine function conjugated to the carbonyl through the benzene ring can act as a powerful alkylating agent [52]. The authors also synthesized 3,3-dimethyl-4-oxo-3, 4-dihydroquinoline **96**, 3,3-dimethyl-4-oxo-2-methoxy-1, 2,3,4- tetrahydroquinoline **97** and 11aS-PBD **98** as models to study the mechanism of action of PBDs. Both compounds **96** and **98** readily add nucleophile to the imine bond but only compound **98**, like the parent antibiotic, readily produces covalent attachment to DNA.

The DNA sequence specificity of the antibiotic has been demonstrated by a footprinting method using methidium propyl-EDTA-iron II [MPE.Fe (II)] [53], and the results show that each of the drugs has a two to three base pair sequence specificity that includes the covalently modified guanine residue, While 5'PuGPu is the most preferred binding sequence for the PBDs 5'PyGPy is the least preferred sequence. Footprinting analysis also reveals a minimum 3 to 4 base pair footprint size for each of the drugs on DNA, with a larger than expected offset (2 to 3 base pairs) on opposite strands.

Anthramycin readily undergoes hydrolytic changes at C11, and the possible relation of this reaction to the interaction with DNA was considered. Both the hydrolysis of the C11-methoxy group and the reaction with DNA were found to be acid catalysed, thus supporting a relation between the two processes. The titration of the proton from the phenolic group at position 9 of anthramycin was found to be absent in the DNA complex, suggesting that position 9 also involved in the complex formation [54].

When anthramycin is recrystallized from hot methanol-water, the anthramycin methylether (AME) is formed. AME inhibits the *in vivo* and *in vitro* RNA and DNA by Ehrlich ascites carcinoma cells. AME is a competitive inhibitor (with respect to DNA) of the cell free synthesis of RNA and DNA by the DNA-dependent polymerase enzymes, as well as the enzymatic hydrolysis of DNA by DNase I. The interaction of AME with DNA was demonstrated by the isolation of an AME-DNA complex by gel filtration, chromatography on Sephadex G-50 columns, by equilibrium dialysis studies, by ultraviolet absorption spectroscopy, by an increase in the melting temparature (T_m) DNA when AME was added, and by the displacement of methyl green complex by AME. The DNA helix must be intact for AME to bind to DNA and for AME to inhibit the DNA-dependent enzymatic reactions. AME-DNA complex prevents DNA from participating as a template in the biosynthesis of RNA and DNA [55]. Chemical and enzymatic probes (hydroxyl radical, DNase I) have been used to evaluate drug sequence-dependent changes in drug-DNA adduct conformation, gel electrophoresis to measure drug-induced bending in DNA, and HPLC to measure the reaction kinetics of anthramycin bonding to different sequences [56]. The results show that tomaymycin bonding to DNA induces greater conformational changes in the DNA (bending associated narrowing of the minor groove) than anthramycin.

The DNA binding sites of Tomamycin (Tma) and Anthramycin (Atm) were identified by exonuclease III (exoIII) digestion, y exonuclease (yExo) digestion and Uvr ABC nuclease incision analysis [57]. Exo III digestion stalls 4-5 bases 3' to a drug-DNA adduct, while this method can recognize most of the Atm and Tma-DNA modification sites, it is completed in that exoIII digestion is also stalled by certain unmodified sequences and by drug bound to the opposite strand.

An HPLC assay was used to study the covalent bonding interaction of carbinolamine containing PBDs with the model nucleophile thiophenol, in order to evaluate

electrophilicity at the C11-position [58]. Preliminary experiments with anthramycin, tomaymycin and neothramycin have shown that their reactions with thiophenol follow second order kinetics, but the order of reactivity (neothramycin > tomaymycin > anthramycin), does not correlate with either *in vitro* cytotoxicity or *in vivo* antitumor activity. This suggests that other factors such as non-covalent DNA interactions play a more crucial role in biological activity than simple alkylating ability.

The molecular mechanics program AMBER, assisted by Chemlab II, was used to model the covalent and non-covalent binding of anthramycin, tomaymycin and neothramycin A to the hexanucleotide conformation [59]. Structures covalently bonded at N2 of guanine gave good fits when placed in either direction in the minor groove.

More intensive study of the interaction of neothramycin with poly[dG-dC] than poly [dI-dC] and poly[dA-dT] suggests that 2-amino group of guanine base of DNA participates in the binding to DNA. The same base specificity has been found in the reaction with 2'-deoxynucleosides. The reaction rate of neothramycin with DNA is slower than those of mitomycin C, actinomycin D and doxorubicin. Amongst the PBD group neothramycin exhibits the slowest reaction rate with DNA. Anthramycin, sibiromycin and tomaymycin possess unsaturated side chains at C2, which may participate in the DNA binding [51]. The absence of a C2 side chain in the neothramycin molecule is probably related to the slow reaction rate with DNA. The lower toxicity of neothramycin in comparison to other PBDs may be due to the slower binding rate to DNA.

The reaction of neothramycin with DNA is acid catalyzed, which may be significant because the acid-base balance tends to be more acidic in cancer cells than in normal cells [60,61]. Neothramycin possesses carbinolamine moieties at both the 3,4- and 10,11- positions, but may be chemically reactive in a similar manner, but the antibiotic binds to DNA mainly through C-11 and 2'-deoxyguanosine. Both C11 and C3 may represent potential sites of reactivity with DNA. However, the steric hindrance of and/or hydrogen bond formation with the double helix DNA may lead to complex formation through C11 more easily than through C3. The characteristics of neothramycin and its reaction mechanism with DNA were studied by fluorescence spectroscopy [62] and its fluorescence intensity is enhanced by the reaction with DNA.

Sibiromycin reacts much faster and more effectively with DNA than either anthramycin or tomaymycin [51]. In fact tomaymycin, that reacts to DNA in the slowest manner, yields the lowest antibiotic to base ratio at saturation and conversely, sibiromycin has the highest antibiotic to base ratio. Anthramycin and sibiromycin are relatively similar in structure except for the aminosugar at C7 of sibiromycin, but exhibit markedly different rates of reaction with DNA. This is very suggestive that the amino sugar of sibiromycin plays a significant role in the interaction of the PBD antitumor antibiotics with DNA.

Biological activity of anhydrosibiromycin and the methylether of sibiromycin were studied [63]. Anhydrosibiromycin, like sibiromycin, formed a complex with DNA and increases the DNA melting temperature but to a lesser extent than sibiromycin. Anhydrosibiromycin exhibited low activity in the system of DNA-dependent RNA polymerase. The low activity of anhydrosibiromycin is due to the instability of the antibiotic with DNA. The methyl ether of sibiromycin had no biological activity and did not interact with DNA. This suggests that the functional groups of the sibiromycin participate in the DNA binding.

The interactions of several PBD with linearized plasmid PGEM-2-N-ras DNA have been analyzed by quantitative *in vitro* transcription (QIVT) and *in vitro* transcription footprinting (IVTF) methods [64]. A concentration-dependent inhibitory effect of the PBDs on transcription is observed by using both the techniques. The order for overall inhibition of transcription by the QIVT method is found to be sibiromycin> tomaymycin> anthramycin> DC-81 > neothramycin, whereas in the IVFT experiments the order is sibiromycin> anthramycin> neothramycin> tomaymycin.

Structural Characterization of Drug-DNA Complexes

NMR Studies

Krugh *et al.*, [65] studied the two-dimensional NMR of an anthramycin DNA adduct d (ATamGCAT) d (ATGCAT). The anthramycin protons in the minor groove exhibit NOEs to several nucleotide protons. The network of anthramycin-nucleotide NOEs and the measurement of the 10-Hz coupling constant between the anthramycin H11 and H11a protons show that anthramycin is covalently attached as the 'S' stereoisomer at the anthramycin C11-position with the side chain of anthramycin oriented toward the 5' end of the modified strand (Fig**. 5**).

Two distinct sets of signals for the tomaymycin molecule are present in the proton NMR spectrum of the tomaymycin-d $(ATGCAT)_2$ duplex adduct [66,67]. Two dimensional correlation spectroscopy (2D-cosy) studies also show connectivity's for four cytosine H5-H6 and eight thymine methyl H6 protons and thus clearly establish the presence of two distinct species of tomaymycin d $(ATGCAT)_2$ adducts in solution. A single scalar 11-11a ^1H NMR coupling in the 2D-COSY spectrum is indicative of an adduct species that has an S configuration at C11-position. Two-dimensional nuclear Overhauser effect (NOESY) spectra of the tomaymycin d $(ATGCAT)_2$ duplex adduct show that the adducts are relatively undistorted. In a NOESY experiment, cross peaks were identified between both the aromatic H9 proton and the ethylidine methyl proton of tomaymycin and two different adenine H2 protons of d $(ATGCAT)_2$. Molecular mechanics calculations with AMBER show that the two species with the thermodynamically most favorable binding energies are the 11R, 11S isomers with their aromatic rings to the 5' and 3' sides of the covalently bound guanine, respectively. The NOEs observed between tomaymycin protons and adenine H2 protons are in accord with molecular model studies. Taken together, these results strongly suggest that the two forms of tomaymycin bound to d $(ATGCAT)_2$ are the 11S, 11aS and 11R, 11aS species, oriented with their aromatic

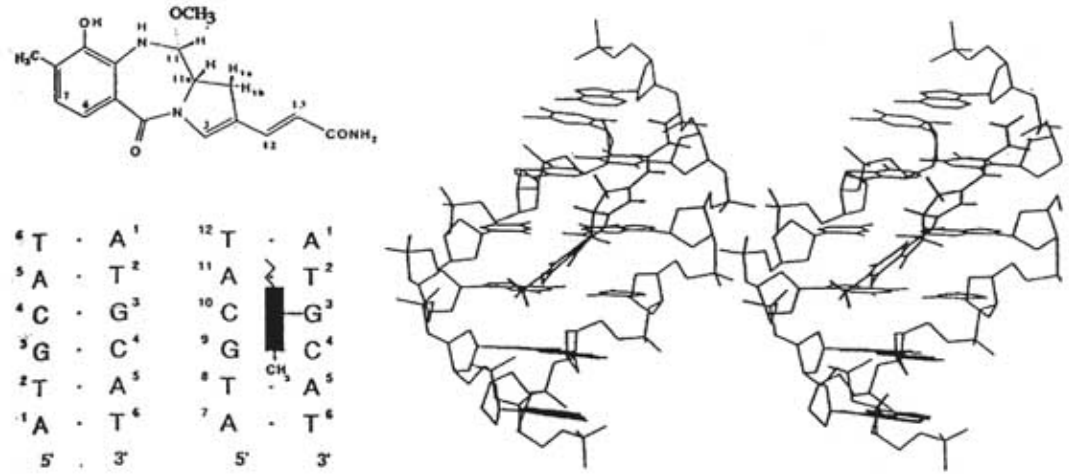


Fig. (5). (Left panels) The chemical structure of anthramycin adduct with d $(ATGCAT)_2$ (bottom). The anthramycin is covalently attached to the 2-amino group of one of the guanines, labeled G3; the modified strand is numbered as nucleotides 1-6. (Right panel) Stereo drawing of the anthramycin-d $(ATGCAT)_2$ adduct. Anthramycin is covalently attached to the exocyclic amino group of G3 by an aminal linkage to C11 of anthramycin. This model is based on the B-form of DNA.

rings to the 3' and 5' sides respectively, of the covalently modified guanines (Fig**. 6**).

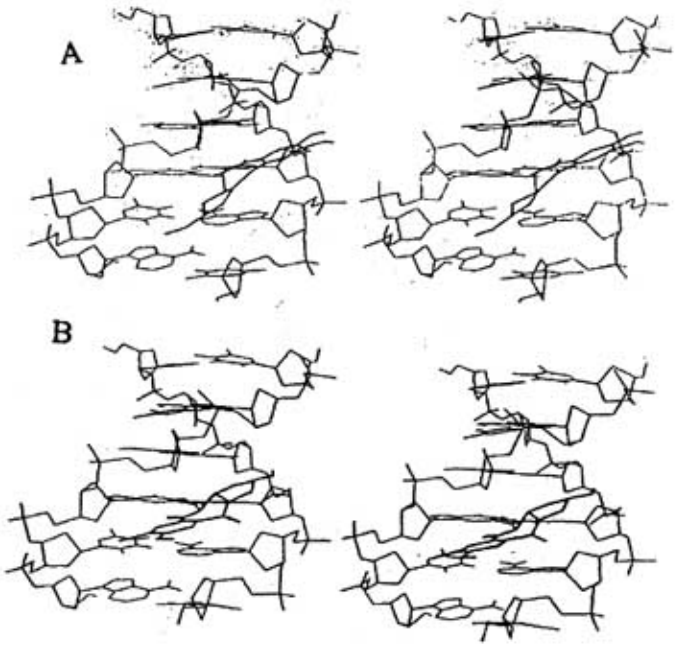


Fig. (6). Stereo diagram of (A) the (11S, 11aS)-tomaymycin-d $(ATGCAT)_2$ duplex and (B) the (11R, 11aS)-tomaymycin-d $(ATGCAT)_2$.

Boyd *et al.*, designed and synthesized a self-complementary 12-mer [d $(CICGAATTCICG)_2$] based on the Dickerson dodecamer [d $(CGCGAATTCGCG)_2$] that bonds identically to two tomaymycin molecules, which has a defined orientation and stereochemistry [68]. Thus the bis-tomaymycin-12-mer adduct maintains the self-complementarity of the original duplex molecule.

In an earlier study [69], it was shown that two tomaymycin molecules can be covalently bound to a 12-mer duplex molecule where the drug molecules are on opposite strands six base pairs apart. Wang *et al.*, in their study using highfield NMR showed that the same 12-mer sequence can be truncated by two base pairs so that the tomamycin-modified guanines are now only four base-pairs apart, the two species of tomaymycin molecules are still bound with the same stereochemistry and orientation, and the 10-mer duplex adduct maintains its self complementarity. In a second 10-mer duplex, they have shown that changing the bonding sequence from 5'-CGA to 5'-AGC does not significantly affect the structure of the bis-tomaymycin-duplex adduct. However, when the sequence was rearranged, so that the drug points in a tail to tail orientation rather than head to head configuration, there were more than one species of tomaymycin bound to DNA, as a consequence, the bis-tomaymycin-10mer duplex adduct loses its self-complementarity (Fig**. 7**).

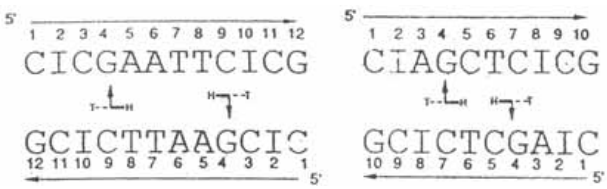


Fig. (7).

The interstrand cross-linked DSB-120 -d $(CICG*ATCICG)_2$ DNA adduct (* indicates covalently modified guanine) was examined by two-dimensional NMR and compared with the bis-tomaymycin adduct on the same oligomer [70]. Tomaymycin and DSB-120 form self-complementary adducts with the d $(CICGATCICG)_2$ duplex sequence in which the covalent linkage sites occur between C11 of either drug and exocyclic 2-amino group of the single reactive guanine on each strand of d

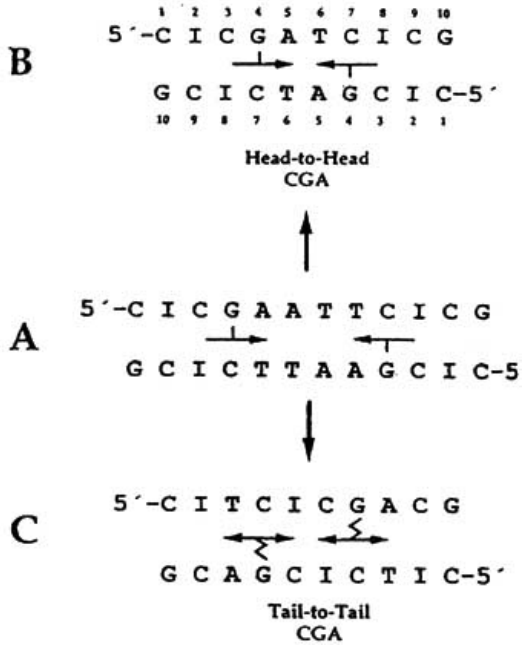


Fig. (8). DNA sequences examined for template-directed bis-tomaymycin alkylation: sequence A, sequence B, and sequence C.

$(CICGATCICG)_2$. In the case of DSB-120, this is evident by the formation of a guanine-guanine interstrand cross-link. Both drugs show formation of S-stereochemistry at the covalent linkage site with an associated 3'-orientation. While the majority of DNA in these adducts appear to be B-form, DSB-120 interstrand cross linking induces typical properties in the 8I nucleotide, indicated by broadening of the 8I H2 proton resonance, non-C2' endo sugar geometry, and usually weak internucleotide NOE connectivity to the 7C nucleotide. Tomaymycin does not produce this regional dislocation. For tomaymycin, while there are strong NOE connectivities from protons on the five membered rings to the 8IH2 proton on the floor of the minor groove, the equivalent internucleotide

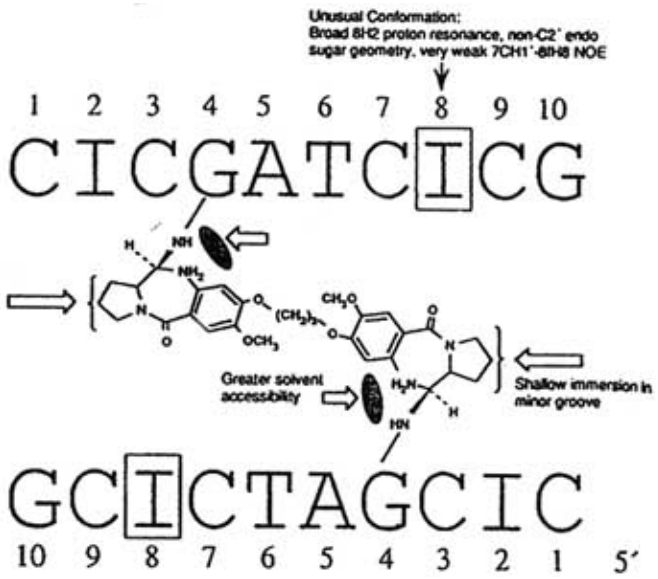


Fig. (9). Summary of unusual properties of the DSB-120 5'CGA cross-linked adducts.

connectivities in DSB-120 are weaker. This indicates that the tomaymycin tail is close to the floor of the minor groove, while the five membered ring of the DSB-120 is more shallowly immersed, perhaps due to strain from cross-linking with a very short linker unit. The conformational stresses induced on the duplex by DSB-120 appear to make the region of covalent attachment more accessible to solvent than is the case for tomaymycin (Fig**. 8 & 9**).

Cellular and Pharmacological Studies

In Vitro Studies

Anthramycin-DNA adducts, produced *in vitro* by reaction of anthramycin with calf thymus DNA, have been shown to be stable only as long as the secondary structure of DNA is maintained [71]. Denaturation either by heat or enzymatic degradation of the DNA adducts, with DNase I and snake venum phosphodiesterase leads to the release of significant amounts of the bound drug as unchanged anthramycin. The ability of the adduct versus free drug to inhibit DNA synthesis and induce unscheduled DNA synthesis in human cell line was evaluated. The results demonstrate that anthramycin DNA-adduct was less potent than the free drug in these systems. The antibacterial activity of 1E and 1Z tomaymycin has been studied by a paper disc method [72]. The 1Z exhibits the same antimicrobial activity as that of naturally occurring 1E tomaymycin.

Neothramycin was observed to prevent growth of lymphoblastoma L5178Y and Hela cells at the concentration of 0.561,0 μg/ml and exhibited a lethal effect at 5.0 μg/ml. Approximately 50% growth inhibition of *E. coli* was found at the concentration of 37 μg/ml [73]. The antibiotic produced a preferential inhibition of RNA over DNA synthesis in the intact cells of lymphoblastoma L5178 Y, *i.e.,* approximately 50% inhibition of the former was observed at the antibiotic concentration of 1.4 μg/ml, and the latter at 12.0 μg/ml.

A series of PBD analogs like tilivalin **67-69** has been synthesized and their cytotoxicity towards mouse leukemia L1210 cells studied [74]. The 11-β cyano compound **67**, a tilivalin analog bearing the cyano group instead of an indole group, is approximately 100 times more cytotoxic than tilivalin **69** itself. The α-epimer **68** of this cyano compound has about one hundredth of the cytotoxicity of the 11 β-cyano compound **67**.

The cellular pharmacology of a series of C8 linked PBD dimers with polymethylene linkers **75-78** has been studied in a range of human tumor cell lines [75]. The four compounds showed the same pattern of relative activity in five ovarian carcinoma cell lines and one cervical carcinoma cell line with the order of IC50 values of compounds **75** <= **77** < **78** < **76**, which correlated with the DNA interstrand cross-linking ability of the compounds in plasmid DNA. In human leukemic K562 cells, these agents produced a block in the G2/M phase of the cell cycle characteristic of cross-linking drugs and extensive interstrand cross-linking was observed in cells by alkaline elution with no evidence of single strand breaks. Cross-links continued to increase up to

24h following a 1h exposure to drug, and no repair was evident by 48h. In a series of ovarian and cervical carcinoma cell lines with acquired resistance to cisplatin no cross link resistance to the most potent compound **75** was observed in two cell lines whose major mechanism of resistance to cisplatin was reduced platinum transport. Cross resistance to **77** was observed in a cell line (A2780 CisR) possessing elevated glutathione, and depletion of intracellular glutathione using D, L-buthionine-S, R-sulfoximine (BSO) from 10.25 mmol to 2-8 nmol 10^{-6} cells reduced the level of resistance from 11-fold to 2-fold compared with sensitive cells. Cross-linking in the resistant cells was restored to 80% of the level in the parent line by BSO pretreatment. There was also a correlation between glutathione levels and sensitivity to **75** measured in several other ovarian cell lines. Compound **75** also showed cross-resistance in the doxorubicin-resistant cell line 41MdoxR and partial cross-resistance in CH1doxR cells.

In Vivo Studies

Anthramycin and the anthramycin-DNA conjugate were compared in mice for lethality, tissue levels, alteration of hexobarbital sleeping times, and efficacy against a mouse ascites tumor model [71]. The results showed that the DNA adducts were three times more lethal and produced similar increase in the sleeping times at equitoxic doses. The increase in lethality of the anthramycin DNA adduct could be explained by elevated and more prolonged blood and tissue levels following administration of the DNA conjugate, compared to free anthramycin, when tested for efficacy against a mouse ascites tumor line. The anthramycin-DNA adduct was found to be less effective than the free drug.

Tomaymycin has potent antitumor activity against L1210 leukemia transplanted intraperitoneally in DBA2/C57 BL mice [76]. In the case of intraperitonial administration of tomaymycin (dose 125 μg/day) in mice for five consecutive days, increase in life span is 26-50%. Tomaymycin shows complete suppression of growth of transplantable ascites tumor such as sarcoma 180 and Ehrlich carcinoma. In the case of intraperitonial administration of tomaymycin (dose 3.13-125 μg/kg/day) for five consecutive days, inhibition of growth is 76-99%. Some of tomaymycin analogs were active against P388 leukemia.

Clinical Studies

In the phase I study of neothramycin a total of 63 cases including 42 various solid tumors and 21 hematologic tumors refractory to standard treatments underwent the study [77]. The most frequent and severest toxicity was nausea and vomiting seen in about half of the patients being administered dosages ranged from 24 mg/m^2 to 40mg/m^2, and three out of four patients received dosages exceeding 50 mg/m^2 required clinical managements. Moreover, one out of two patients administred 60 mg/m^2 were ranked as grade four of the criterion of toxicities. Other clinical toxicities such as skin rash, hepatotoxicity or nephratoxicity observed in a minority of patients were reversible. Furthermore, hematologic toxicity was extremely mild and appeared not to be dose dependent. One patient with chronic myelogeneous leukemia had a hematological improvement and the other with esophageal cancer had a partial response. The results indicate that a dose-limiting factor of neothramycin is nausea and vomiting, and a maximum tolerated dose of a single injection is 60 mg/m^2. A dose schedule of 30-40 mg/m^2 appears to be an optional dose for phase II study.

Neothramycin has been used in treatment of superficial carcinoma of the bladder [78]. Neothramycin was instilled into the bladder in the following dosages; 10mg neothramycin in 20 mL of sterile distilled water was given first, and increased to 40mg in 20ml. This procedure was performed every second week to twice a day. In four of eleven patients (36%) the tumors disappeared completely, while in six patients (55%) there was partial disappearence of more than 50% and in one patient (9%) there was no effect. Neothramycin was thus concluded to be effective for superficial carcinoma of the bladder.

CONCLUSIONS AND PROSPECTS

With better understanding of the role of nucleic acids-protein interactions in the regulation of gene expression, nucleic acids have become prime targets for the development of new compounds that are able to control gene expression. In the past decades, tremendous efforts have been made to develop drugs, which are expected to interfere with the replication and transcription of DNA by binding to double-stranded DNA in a sequence specific manner, and have applications in the field of cancer. In the last few years a variety of polyamide conjugates has been synthesized by combining polyamide with other DNA effectors such as intercalators, strand cleavage agents, including bleomycin analogs, enediyenes and alkylating agents, such as CPI, CBI, and PBDs. A number of PBD analogues have been synthesized, but the biological studies for few of these synthetic analogues have been carried out to date. A new class of PBD- polyamide conjugates may posses high sequence selectvity and this may enhance the alkylation property of the PBDs.

ACKNOWLEDGEMENT

The research was supported by a grant (to J.W.L.) from the Natural Sciences and Engineering Research Council of Canada.

ABBREVIATIONS

AME = Anthramycin methylether

DNA = Deoxyribonucleic acid

EDTA = Ethylenediamine tetraacetic acid

FPA = Fluorescence polarization anisotropy

FTIR = Fourier transform infrared spectroscopy

HIV = Human immunodeficiency virus

ISC = Interstrand crosslinks

NMR = Nuclear magnetic resonance

NOE = Nuclear Overhauser effect

PBD = Pyrrolo [2,1-*c*][1,4] benzodiazepine

RNA = Ribonucleic acid

REFERENCES

[1] Lemgruber, W.; Batcho, A.D.; Schenker, F. *J. Am. Chem. Soc*. **1965**, *87*, 5793.; Lemgruber, W.; Stefanovic, V.; Schenker, F.; Karr, A.; Berger, J. *J. Am. Chem. Soc*. **1965**, *87*, 5791.

[2] Kunimoto, S.; Masuda, T.; Kanbayash, N.; Hamada, M.; Naganawa, H.; Miyamoto, M.; Takenchi, T.; Umezawa, H. *J. Antibiot*. **1980**, *33*, 665.

[3] Shimizu, K.I.; Kawamoto, I.; Tomita, F.; Morimoto, M.; Fujumoto, K. *J. Antibiot.* **1982**, *35*, 972.

[4] Arima, K.; Kohsaka, M.; Tamura, K.G.; Imanaka, H.; Sakai, H. *J. Antibiot.* **1972,** *25*, 437.

[5] Hara, M.; Tamaoki, T.; Yoshida, M.; Morimoto, M.; Xlakano, H. *J. Antibiot*. **1988**, *41*,702.

[6] Itoh, J.; Watanabe, H.O.; Ishii, S.; Gomi, S.; Nagasawa, M.; Yamamoto, A. *J. Antibiot*. **1988**, *41*, 1281.

[7] Takeuchi, T.; Miyamoto, M.; Ishizuka, M.; Naganawa, H.; Kondo, S.; Hamada, M.; Umezawa, H. *J. Antibiot.* **1976**, *29*, 93.

[8] Thurston, D.E. *Molecular Aspects of Anticancer Drug-DNA Interactions* **1993**, *1*, 54.

[9] Thurston, D.E.; Murthy, V.S.; Langley, D.R.; Jones, G.B. *Synthesis* **1990**, 81.

[10] Gauze, G.F.; Preobrazhenskaya, T.P.; Ivanitskaya, L.P.; Sveshnikova, M.A. *Antibiot*. **1969**, *14*, 963.

[11] Knoishi, M.; Ohkuma, H.; Naruse, N.; Kowaguchi, H. *J. Antibiot*. **1984**, *37*, 200.

[12] Hoschlowski, J.E.; Andrews, W.W.; Theriavit, R.J.; Jackson, M.; McAlpine, J.P. *J. Antibiot*. **1987**, *40*, 145.

[13] Miyamoto, M.; Kondo, S.; Naganawa, H.; Maeda, K.; Ohno, M.; Umezawa, H. *J. Antibiot.* **1977**, *30*, 340.

[14] Kohn, K.W.; Bono, V.H.Jr.; Kann, H.E. Jr. *Biochem. Biophys. Acta* **1968**, *155*, 121.

[15] Bose, D.S.; Jones, G.B.; Thurston, D.E. *Tetrahedron* **1992**, *48*, 751.

[16] Thurston, D.E.; Bose, D.S. *Chem. Rev.* **1994**, *94*, 433.

[17] Baraldi, P.G.; Cacciari, B.; Guiotto, A.; Romagnoli, R.; Spalluto, G.; Zaid, A.N.; Cozzi P.; Geroni, C.; Mongelli, N. *Farmaco*, **1997**, *52*, 711.

[18] Kamal, A.; Rao, M.V.; Laxman, N.; Ramesh, G.; Reddy, G.S.K. *Current Medicinal Chemistry-Anti-Cancer Agents* **2002**, *2*, 215.

[19] Jones, G.B.; Davey, C.L.; Jenkins, T.C.; Kamal, A.; Kneale, G.G.; Neidle, S.; Webster, G.D.; Thurston, D.E. *Anti-Cancer Drug Design* **1990**, *5,* 249.

[20] Gillard, A.C.; Rault, S.; Boulourad, M.; Robba, M.J. *Heterocyclic Chem.* **1996**, *33*, 275.

[21] Boulourad, M.; Gillard, A.C.; Guillaumat, P.O.; Daoust, M.; Legrand, E.; Quermonne, M.A.; Rault, S. *Pharm. Pharmacol. Commun.* **1998**, *4,* 43.

[22] Farmer, J.D. Jr.; Gustafson, G.R.; Conti, A.; Zimmt, M.B.; Suggs, J.W. *Nucleic acids Res*. **1991**, *19*, 899.

[23] O'Neil, I.A.; Murray, C.L.; Hunter, R.C.; Kalindjian, S.B.; Jenkins, T.C. *Syn. Lett.* **1997**, 75.

[24] Thurston, D.E.; Jones, G.B.; Davis, M.E. *J. Chem. Soc. Chem. Commun.* **1990**, 874.

[25] Gillard, A.C.; Rault, S.; Boulouard, M.; Robba, M. *J. Heterocyclic Chem.* **1995**, *32*, 1741.

[26] Langlois, N.; Adriamialisoa. *Heterocycles* **1989**, *29*, 1529.

[27] Kamal, A.; Reddy, B.S.P.; Thurston, D.E. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 743.

[28] Mori, S.; Ohno, T.; Harada, H.; Aoyama, T.; Shioiri, T. *Tetrahedron* **1991**, *47*, 5051.

[29] Shiori, T.; Aoyama, T.; Yamagami, N.; Shimizu, N.; Mori, N.; Kohda, K. *Anti-Cancer Drug Design* **1995**, *10*, 167.

[30] Baraldi, P.G.; Leoni, A.; Cacciari, B.; Manfredini, S.; Simoni, D.; Bergomi, M.; Menta, E.; Spinellis, S. *J. Med. Chem.* **1994**, *37***,** 4329.

[31] Fouchet, S. J.; Fabis, F.; Bovy, P.; Ochseenbein, P.; Roult, S. *Tetrahedron Letters* **1998**, *39*, 819.

[32] Gillard, A.C.; Fabis, F.; Fouchet, S.J.; Roult, S. *Tetrahedron Letters* **1997**, *38*, 2271.

[33] Prabhu, K. R.; Sivanand, P. S.; Chandrsekaran, S. *Syn. Lett.* **1998**, 47.

[34] Kamal, A.; Reddy, B. S. P.; Reddy, B. S. N. *Tetrahedron Letters* **1996**, *37*, 6803.

[35] Kamal, A.; Reddy, B. S. N.; Reddy, B. S. P. *Bioorg. Med. Chem. Lett*. **1997**, *7,* 1825.

[36] Kamal, A.; Damayanthi, Y.; Reddy, B. S. N.; Lakminarayan, B.; Reddy, B. S. P. *Chem. Commun.* **1997**, 1015.

[37] Kamal, A.; Laxman, E.; Reddy, P. S. M. M. *Tetrahedron Letters* **2000**, *41*, 8631.

[38] Kamal, A.; Laxman, E.; Venugopal Rao, N. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 2311.

[39] Bose, D.S.; Thompson, A.S.; Ching, J.; Hartely, J.A.; Berardini, M.D.; Jenkins, T.C.; Neidle, S.; Hurley, L.H.; Thurston, D.E. *J. Am. Chem. Soc*. **1992,** *114*, 4939.

[40] Bose, D.S.; Thompson, A.S.; Smellie, M.; Berardini, M.D.; Hartely, J.A.; Jenkins, T.C.; Nediel, S.; Thurston, D.E. *J. Chem. Soc. Chem. Commun*. **1992**, 1518.

[41] Thurston, D.E.; Bose, D.S.; Thompson, A.S.; Howard, P.W.; Leoni, A.; Croker, S.J.; Jenkins, T.C.; Neidle, S.; Hartely, J.A.; Hurley, L.H. *J. Org. Chem*. **1996**, *61*, 8141.

[42] Baraldi, P. G.; Cacciari, B.; Guiutto, A.; Leoni, A.; Romagnoli, R.; Spalluto, G.; Mongelli, N.; Howard, P.; Thurston, D.E.; Bianchi, N.; Gambari, R. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 3019.

[43] Damayanthi, Y.; Reddy, B. S. P.; Lown, J. W. *J. Org. Chem*. **1999**, *64*, 290.

[44] Reddy, B. S. P.;Damayanthi, Y.; Lown, J. W. *Syn. Lett*. **1999**, 1112.

[45] Reddy, B. S. P.; Damayanthi, Y.; Reddy, B. S. N.; Lown, J. W. *Anti-Cancer Drug Design* **2000**, *15*, 225.

[46] Kumar, R.; Reddy, B. S. N.; Lown, J. W. *Heterocyclic Communication* **2002**, *8*, 19.

[47] Kumar, R.; Lown, J. W. *Heterocyclic Communication* **2002**, *8*, 115.

[48] Hurley, L.H.; Petrusek, R. *Nature* **1979**, *282*, 529.

[49] Hurley, L.H.; Rokem, J.S.; Petrusek, R.L. *Biochem. Pharmacol*. **1980**, *29*, 1307.

[50] Petrusek, R.L.; Anderson, G.L.; Garner, T.F.; Fannin, Q.L.; Kalpan, D.J.; Zimmer, S.G.; Hurley, L.H. *Biochemistry* **1981**, *20*, 1111.

[51] Hurley, L.H. *J. Antibiot*. **1977**, *30*, 349; Hurley, L.H.; Gairola, C.; Zmijewski, M. *Biochim. Biophys. Acta* **1977**, *475*, 521.

[52] Lown, J.W.; Joshua, A.V. *Biochem. Pharmacol.* **1979**, *28*, 2017.

[53] Hertzberg, R.P.; Hecht, S.M.; Reynolds, V.L.; Molinewe, I.J.; Hurley, L.H. *Biochemistry* **1986**, *25*, 1249.

[54] Glaubiger, D.; Kohn, K.W.; Charney, E. *Biochim. Biophys. Acta* **1974**, *361*, 303.
[55] Bates, H.M.; Kuenzig, W.; Watson, W.B. *Cancer Res.* **1969,** *29*, 2195.
[56] Kizu, R.; Draves, P.H.; Hurley, L.H. *Biochemistry* **1993**, *32*, 8712.
[57] Pierce, J.R.; Zazimiec, M.; Tang, M.S. *Biochemistry* **1993**, *32*, 7069.
[58] Morris, S.J.; Thurston, D.E.; Nevell, T.G. *J. Antibiot.* **1990**, *43*, 1286.
[59] Remers, W.A.; Mabilia, M.; Hopfinger, A.J. *J. Med. Chem.* **1986**, *29*, 2492.
[60] Ross, W.C.J. *Biochem. Pharmacol.* **1961**, *8,* 235.
[61] Connors, T.A.; Mitchley, B.C.V.; Rosenoer, V.M.; Ross, W.C.J. *Biochem. Pharmacol*. **1964**, *13*, 395.
[62] Maruyama, I.N., Tanaka, N., Kondo, S.; Umezawa, H. *J. Antibiot.* **1981**, *34*, 427.
[63] Koz'mian, L.I.; Dudnik, Lu.V.; Shepelevtseva, N.G. *Antiobiot*. **1977**, *22*, 602.
[64] Puvvada, M.S.; Forrow, S.A.; Hartley, J.A.; Stephenson, P.; Gibson, I.; Jenkins, T.C.; Thurston, D.E. *Biochemistry* **1997**, *36*, 2478.
[65] Cheatham, S.; Kook, A.; Hurley, L.H.; Barkley, M.D.; Remers, W. *J. Med. Chem*. **1988**, *31* 583.
[66] Barkley, M.D.; Cheatham, S.; Thurston, D.E.; Hurley, L.H. *Biochemistry* **1986**, *25***,** 3021.
[67] Boyd, F.L.; Stewart, D.; Remers, W.A.; Barkley, M.D.; Hurley, L.H. *Biochemistry* **1990,** *29*, 2387.
[68] Wang, J.J.; Hill, G.C.; Hurley, L.H. *J. Med. Chem.* **1992**, *35*, 2995.
[69] Mountzouris, J.A.; Wang, J.J.; Thurston, D.E.; Hurley, L.H. *J. Med. Chem.* **1994**, *37*, 313.
[70] Kopka, M.L.; Goodsell, D.S.; Baikalov, I.; Grzeskowiak, K.; Cascio, D.; Dickerson, R.E. *Biochemistry*, **1994**, *33*, 13593.
[71] Hurley, L.H.; Allen, C.S.; Feola, J.M.; Lubawy, W.C. *Cancer Res*. **1979**, *39*, 3134.
[72] Tozuka, Z.; Takasugi, H.; Takaya, T. *J. Antibiot.* **1983**, *36*, 276.
[73] Maruyama, I.N.; Suzuki, H.; Tanaka, N. *J. Antibiot.* **1978**, *31*, 761.
[74] Smellie, M.; Kelland, L.R.; Thurston, D.E.; Souhami, R.L.; Hartley, J.A. *Br. J. Cancer* **1994**, *70*, 48.
[75] Tozuka, Z.; Yazawa, H.; Murata, M.; Takaya, T. *J. Antibiot.* **1983**, *36*, 1699.
[76] Kimura, K.; Ogawa, M.; Wakui, A.; Oguro, M.; Koyama, Y.; Saito, T.; Furue, H.; Ota, K.; Yamada, K.; Hoshino, A.; Nakamura, T.; Masaoka, T.; Taguchi, T.; Kimura, I.; Hattori, T. *Gan to kagaku Royoho*, **1982**, *9*, 924.
[77] Tsugaya, M.; Washida, H.; Hirao, N.; Hachisuka, Y.; Sakagami, H.; Iwase, Y. *Hinyokika Kiyo-Acta Urologica Japanica* **1986**, *32*, 1443.
[78] Krugh, T.R.; Graves, D.E.; Stone, M.P. *Biochemistry* **1989**, *28*, 9988.