Naturally Occuring Pyrrolo[1,4]benzodiazepines in Bacteria

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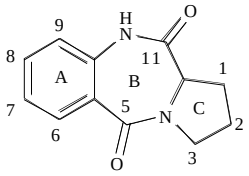
Abstract: Pyrrolo[1,4]benzodiazepines (PBDs) are a growing group of microbial secondary metabolites that showed remarkable biological activities particularly against tumor cells. Their antitumor activity is mainly due to their specifically binding to DNA and other polydeoxynucleotides to form irreversibly polymer-bound drugs. Consequently, they have been the target of several syntheses as well as screening of natural sources to discover new members. This review provides an update on recent discoveries of PBDs as well as a compilation of spectroscopic data described in the literature.

Keywords: Pyrrolo[1,4]benzodiazepines (PBDs), biological activity, natural products.

1. INTRODUCTION AND GENERALITIES

The pyrrolo[1,4]benzodiazepines (PBDs) group of natural products is a small class of secondary metabolites isolated from various microorganisms (bacteria, fungi) and myxomycetes. To date, approximately 32 structures have been isolated and reported from bacteria. Anthramycin, the first member of the PBD family, was first isolated in 1965 by Leimgruber *et al.* [1, 2]. The PBDs possess a tricyclic structure composed of a di, tri, tetra, or in rare cases pentasubstituted aromatic ring A, a 1,4-diazepandione ring system B and a five member carbon skeleton ring C.

In general, the tricyclic moiety of anthramycin is present in all natural PBDs. The most distinctive differences include (i) substitution patterns on the aromatic ring which occurs especially at C-7, C-8 and C-9; (ii) the presence or absence of the C-11 carbonyl; (iii) the presence of the imine bond between N-10-C-11 and substitution at C-11 of the B-ring [3, 4]. Three major modifications are also observed for the five membered pyrrolidine ring: (1) substitution at C-2 or C-3; (2) saturated or unsaturated bond at C-2-C-3 (endocyclic) or C-2-C-12 (exocyclic), and in rare case (3) a fully unsaturated pyrrolidine ring (pyrrole). Compounds with no substituent at C-2, e.g; the antibiotic DC-81 [5], have also been isolated and reported.



Various substituents, such as hydroxyl and alkyl groups have been observed at the C-2 position of the five member ring C. Alkylation at C-2 may be either acrylamide (anthramycin) [1], *N*-methyl acrylamide (mazethramycin) [6, 7], *N,N*-dimethyl acrylamide (porothramycin) [8], ethylidene (tomaymycin)[9], propylidene (sibiromycin) [10], or ethyl group (limazepines) [11]. Hydroxylation at C-2 is only reported in chicamycins A and B [12].

Substitutions on the aromatic ring vary from groups such as hydroxy, methoxy, *O*-glycosyl to methyl, with a number of substitution patterns such as 8-methyl-9-hydroxy (anthramycin, mazethramycin), 7-methoxy-8-hydroxy (tomaymycin, chicamycins, neothramycins, RK-1441A and B, SE15), 8-methoxy-9-hydroxy (limazepines), 9-hydroxy (tilivalline), 9-methoxy (porothramycin), 7-sibirosaminide (DC-102, sibanomicin) and 7-sibirosaminide-8-methyl-9-hydroxy (sibiromycin). Among them are the only three glycosylated PBDs reported so far. Sibiromycin, sibanomicin, and DC-102.

The PBDs are secondary metabolites with relevant activities such as antibacterial, antiviral and most importantly antitumor activities [10,13,14]. However, they exhibited weak or no *in vivo* chemotherapeutic activity against bacterial, viral, fungal, protozoal, and helmintic infections [15]. Due to their potent antitumor activity, PBDs have been popular targets in the synthetic community and number of their derivatives have been prepared [16-19] and tested. Among the synthetic compounds, SJ-136 was reported to be active against a number of human cancer cell lines including cisplatin-resistant ovarian cancers and is currently under clinical trial [20].

Biosynthetic studies using radioisotope labelling experiments have shown that a number of PBDs such as anthramycin, tomaymycin and the aglycone of sibiromycin are derived from thryptophan, tyrosine and methionine [10, 21] and recently the biosynthetic gene cluster of anthramycin have been reported [22]. This review describes the classification of various bacterial pyrrolo[1,4]benzodiazepines and summarises their physico-chemical properties including some NMR data.

1.1. History and Current Status

Anthramycin, the first member of the PBD family of natural products, was isolated from *Streptomyces refuineus* var. *thermotolerans* by bio-guided fractionation method using *Sarcina lutea* (PCI-1001) and *Bacillus* sp. TA (NRRL B-3167) as test organisms [1, 2]. Anthramycin and its derivatives are very labile substances which are difficult to characterize. They are extremely sensitive to heat and are stable in solution only under essentially neutral conditions [2]. Several studies have shown that anthramycin exhibited *in vivo* cytotoxic activity as well as antitumor activity against transplantable tumors in mice. However, its cardiotoxicity prevents its further development [23]. The stereochemistry in anthramycin was first established based on NMR data [1] and later by total synthesis [24].

1.2. Sources

Most of the reported pyrrolo[1,4]benzodiazepines (PBDs) have been isolated from microorganisms especially those of the genus *Streptomyces* (see Table **1**). Antitumor and antibacterial activities were used to screen those organisms and bioassay-guided fractionation technique was employed for their isolation. Fermentation of these Streptomycetes were carried out in complex media containing nutrient such as $CaCO_3$, NaCl, meat, yeast extract, glycerol, soybean meal and corn starch on rotary shaker or jar fermenter at 28°C for 3-7 days.

The fermentation broth is either decanted after centrifugation or filtered to separate the mycelium from the broth. The liquid broth is either absorbed on Amberlite XAD-4 [25], Diaion HP-20 [10], or extracted with organic solvent such as n-butanol. In several cases

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Table 1. Pyrrolo[1,4]benzodiazepines Producing Organisms

Compounds	Organisms	Screening Assay
Abbeymycin (1)	*Streptomyces* sp. AB-999F-52	-
Anthramycin (2)	*Streptomyces refuineus* subsp. *thermotolerans*	Antibacterial
Chicamycin A (3)	*Streptomyces albus* No. J576-99 (ATCC 39143)	-
Chicamycin B (4)	*Streptomyces albus* No. J576-99 (ATCC 39143)	
DC-81 (5)	*Streptomyces roseiscleroticus*	-
DC102 (6)	*Streptomyces* sp.	Antibacterial
Limazepines A-F (7-12)	*Micrococcus* sp.	Antibacterial
Mazethramycin (13)	*Streptomyces thioluteus* ME561-L4	Antibacterial
Nethramycin A (14)	*Streptomyces* No. MC916-C4	Antibacterial
Nethramycin B (15)	*Streptomyces* No. MC916-C4	Antibacterial
Oxotomaymycin (16)	*Streptomyces achromogenes var. tomaymyceticus*	-
Porothramycin A(17)	*Streptomyces albus*	Antibacterial
Porothramycin B(18)	*Streptomyces albus*	Antibacterial
Prothracarcin (19)	*Streptomyces umbrosus*	-
RK-1441A (20)	*Streptomyces* sp. RK-1441	Antibacterial
RK-1441 B (21)		
SEN215 (22)	*Streptomyces cylindrosporus S.E.N.-315, S. sakayensis)*	-
SA4-3 (23)	*Streptomyces actamyceticus*	-
Sibanomicin (24)	*Micromonospora* sp. SF2364	Antibacterial
Sibiromycin (25)	*Streptosporangium sibiricum*	Antibacterial
Compound YM (26)	*Streptomyces* sp. YM8-053	-
Tilivalline (27)	*Klebsiella pneumoniae* var. *oxytoca*	-
Tomaymycin (28)	*Streptomyces achromogenes* var. *tomaymyceticus*	Antibacterial
Tomaymycin I (29)	*Nocardia sp.* C-15003 (ATCC 31281)	-
11-demethyl Tomaymycin (30)	*Streptomyces achromogenes* var. *tomaymyceticus*	-

the PBDs metabolites have been found to be present in both the broth and the mycelium cake. Conventional separation methods like Sephadex LH-20, silica gel, ion exchange DEAE-Sepharose CL-6B (Cl^-) column chromatography as well as preparative HPLC were used for their purification.

2. PHYSICOCHEMICAL PROPERTIES OF PBDS

Most PBDs appear as yellowish to colourless amorphous powder. Their structures are reported to be unstable easily and interconvert from the imine form to the hydroxy or alkyl ether and *vice versa* [2, 26]. It has been demonstrated that, in some cases, this interconvertion depends upon the isolation procedures [10]. The structure of PBDs are generally determined using spectroscopic methods and total synthesis. All PBDs are structurally related and these similarities facilitate their structures elucidation. A look in published PBDs data revealed that they possessed a positive optical rotation (Table **2**); their UV spectra generally indicated three maxima between 210-340 nm, and no report have been published about the influence of substituent on the UV data.

The proton NMR spectra of PBDs are generally less complex; the signals splitting in the aromatic region depend upon the substitution pattern on the ring. Aromatic signals coupling are generally important for the classification of various PBDs groups. Some PBDs compounds possess imine or aminal groups at C-11, the proton chemical shift of imine group generally appears between δ 7.60-8.00. Signal of H-11a appears usually at δ 3.80-4.50. The absence of signal due to H-11a proton is observed in all PBDs with aromatised pyrrolidine ring (pyrrole). The ^{13}C NMR data of PBDs is also very important in their structure elucidation. It indicated characteristic signal of amides C-5 and C-11 at δ 152-171 and δ 165-168 respectively. For the NMR data of some PBDs derivative see Tables **3-7**.

2.1. Chemical Diversity of PBDs

The pyrrolo[1,4]benzodiazepines can be classified in four classes based on the substitution pattern on the aromatic ring. The four classes are: (1) the unsubstituted aromatic ring, (2) monosubstituted (9-hydroxy/methoxy, 7-*O*-sugar), (3) disubstituted (8-methyl-9-hydroxy, 7-methoxy-8-hydroxy) and (4) trisubstituted (7-*O*-sugar-8-methyl-9-hydroxy). To date no PBDs have been reported possessing a fully substituted aromatic ring.

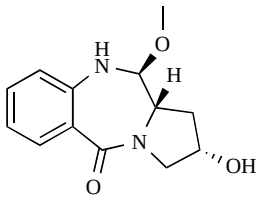
2.2. Unsubstituted Aromatic Ring

They are only few reports on PBDs possessing unsubstituted aromatic ring reported in the literature. These include abbemycin, limazepine F and prothracarcin. Abbeymycin which was isolated from *Streptomyces* sp. AB-999F-52, possess a stereochemistry at C-11 opposite to the other PBDs. Abbeymycin is reported to exhibit only weak antibacterial activity [25]. Interestingly, compound SA4-3 isolated and from *Streptomyces actamyceticus* MS4-3 [27], was reported to have the same planar structure as abbemycin, however,

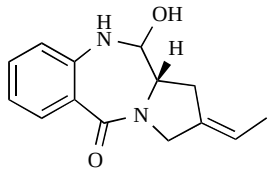
Table 2. Characteristic Data of Some Natural PBDs

Compounds	Melting Point (°C)	$[\alpha]_D$	UV: λ_{max} nm (ε) and (log ε) for Limazepines
Abbeymycin (**1**)	142-144	+303 ($_C$, 0.741, H_2O)	216 (37,200), 236 (18,100) 316 (3,600)[a]
Anthramycin (**2**)	120	+930 (*c* 1.00, DMF).	235 (18,200), 333 (31,800) [b]
Chicamycin A (**3**)	161-163	+350 ($_C$, 0.5, pyr)	232 (24,900), 260 (sh. 7,700), 320 (3,900)[b]
Chicamycin B (**4**)	134-136	+552 ($_C$, 0.5, pyr)	232 (20,200), 260 (sh. 6,900), 318 (3,000)[b]
DC-102 (**5**)	120	-	210 (14,000), 244 (sh, 11,000), 310 (6,500)[c]
DC-81 (**6**)	130-132	+171.5 (*c* 0.1, CH_2Cl_2)	-
Limazepine A (**7**)	-	+ 480 (*c* 0.1, MeOH)	303 (3.95), 280 (3.84), 265 (3.91) [c]
Limazepine B (**8**)	-	-	-
Limazepine C (**9**)	-	+ 160 (*c* 0.4, MeOH)	280 (4.00), 235 (4.09), 210 (4.07) [c]
Limazepine D (**10**)	-	-	403 (sh), 391 (3.46), 289 (3.80), 233 (sh) [c]
Limazepine E (**11**)		+ 73 (*c* 0.6, MeOH)	302 (3.57), 269 (3.84), 235 (3.94), 212 (3.93) [c]
Limazepine F (**12**)	-	260 (*c* 0.06, MeOH)	312 (3.26), 226 (3.93) [c]
Mazethramycin (**13**)	181-193	+730 (*c* 0.069, DMF)	320 (sh.34,600), 335 (39,400)[b]
Mazethramycin methyl ether	216-233	+450 (*c* 0.067, DMF)	217 (25,700), 235 (sh. 19,300), 333 (43,600)[b]
Neothramycin A (**14**)	132-147	+272 (*c* 0.52, dioxane)	223 (sh 22,400), 240 (sh), 265 (7,600), 318 (4,100) [a]
Neothramycin B (**15**)	144-151	+314 (*c* 0.48,dioxane)	224 (24,200), 240 (sh.), 265 (sh.), 318 (4,380)[a]
Porothramycin A (**17**)	140-150	+432 (*c* 0.46, $CHCl_3$)	214 (22,000), 235 (20.300), 335 (45,500)[c]
Porothramycin B (**18**)	164-166	+606 ($_C$, 0.62, $CHCl_3$)	213 (27,800), 236 (27,100) 338 (57,500)[c]
Prothracarcin (**19**)	85-87	+17.1 (*c* 0.1, EtOAc)	218 (21,000), 239 (sh 11,000), 255 (sh 7,000), 316(4,000)[c]
RK-1441A (**20**)	115-120	+692 (*c* 0.52, MeOH)	235 (31,000), 264 (11,900), 318 (5,900)[d]
RK-1441B (**21**)	224-235	+219 (*c* 0.54, EtOH)	235 (55,000), 267 (sh 15,400), 300 (8,600), 310 (sh, 6,600)[d]
Sibanomicin (**24**)	-	+371 (*c* 0.2, DMSO)	214, 240, 290, 314[a]
Sibiromycin (**25**)	120	+525 (*c* 0.35, DMF)	230 (25,950), 310 (21,800)[c]
Tillivalline (**27**)	180-185	+210 (*c* 1.01, MeOH)	
Tomaymycin (**28**)	120	+930 (*c* 1.00, DMF)	224 (36,000), 237 (30,000), 260 (9,000) 320 (3,600)

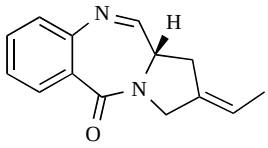
[a]H_2O; [b] MeCN; [c]MeOH; [d]50%EtOH



Abbemycin



Limazepine F



Prothracarcin

no stereochemistry have been indicated for the antibiotic SA4-3. Other members of the unsubstituted PBDs include prothracarcin, isolated from *Streptomyces umbrosus* [28] and *Micrococcus* sp. and limazepine F, recently isolated from *Micrococcus* sp. The latter compound exhibited no antibacterial activity.

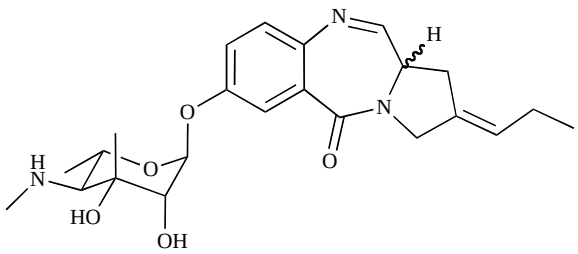
2.3. Monsubstituted Aromatic Ring

Substitution on aromatic ring in natural PBDs occurred mainly at C-7, C-8 or C-9. The positions C-7 and C-9 are generally oxygenated in form of their hydroxy, methoxy or *O*-glycosyl groups. The latter is so far reported only at C-7. Porothramycins A and B, are the only naturally occurring PBDs that possess the *N*,*N*-dimethyl propenamide side chain and a methoxy at C-9. Porothramycin B was described as an artefact because it can only be isolated when methanol was used as solvent during the purification process. Both compounds share the same configuration with anthramycin. Porothramycins A and B exhibited a potent antitumor activity against leukemia P388 and L1210 cells, but were marginally effective against melanoma B16 [10] cells.

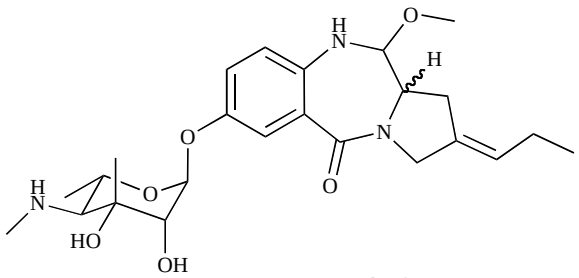
Tilivalline, isolated from *Klebsiella pneumoniae* var. *ocytoca*, is the only example of natural PBDs *C*-substituted at C-11 with an indol moiety. In addition, tilivalline possess the C-11 *R* configuration, which is less common in PBDs, and the C-11a *S* configuration as found in all other PBDs. Sibanomicin [29] and DC-102 [30, 31], isolated respectively from *micromonospora* sp. and S*treptomyces*, are among the rare PBDs reported so far with *O*-glycosylation at C-7 and a propylidene moiety at C-2 of the ring C. The aminosugar moiety sibirosaminide was identified by chemical degradation [32, 33]. The sugar linkage was found to be α [31]. Although the branched olefin at C-2 and C-12 positions was confirmed to have *E* configuration in sibanomicin, the absolute configuration at C-11a is still unknown in these compounds.

2.4. Disubstituted Aromatic Group

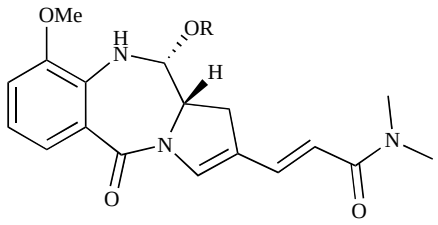
Disubstituted PBDs group could be divided in three sub-classes based on the substitution pattern and their nature: the 8-methyl-9-hydroxy**,** the 7-methoxy-8-hydroxy and the 8-methoxy-9-hydroxy substitutents.



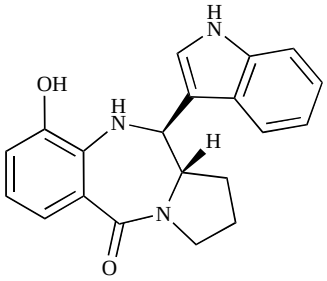
Sibanomicin



DC-102



Porothramycin A: R = H
Porothramycin B: R = CH_3

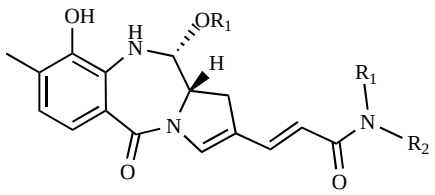


Tilivalline

2.4.1. 8-methyl-9-hydroxy Substituted Aromatic Ring

The antitumor anthramycin is the first member of this group; its structure was determined by interpretation of the NMR spectroscopy. The relative configuration of the two chiral centers at C-11 and C-11a was first assigned through a combination of NMR spectroscopy and total synthesis [1, 24], and the absolute configuration was confirmed by X-ray crystallography [34].

Mazethramycin the methyl amide derivative of anthramycin is isolated from the culture broth of *Streptomyces thioluteus* ME561-L4 and reported to possess antibacterial activities. The methyl and ethyl ether derivatives of mazethramycin were obtained by treatment of the parent compound with anhydrous methanol or ethanol. Compounds from this group possess a substituent at C-11 and a propenamide side chain at C-2 of the pyrrolidine ring.



Anthramycin: $R_1 = R_2 = R_3 = H$
Mazethramycin: $R_1 = R_2 = H$, $R_3 = CH_3$
Mazethramycin methyl ether: $R_1 = CH_3$, $R_2 = H$, $R_3 = CH_3$
Mazethramycin ethyl ether: $R_1 = Et$, $R_2 = H$, $R_3 = CH_3$

2.4.2. 7-Methoxy-8-hydroxy Substituted Aromatic Ring

7-Methoxy-8-hydroxy substituted aromatic ring containing PBDs is the largest class of PBD natural products. Tomaymycin isolated from *Streptomyces achromogenes* var. *tomaymyceticus*, is the first member of this group. The absolute stereochemistry at C-11 and C-11a was determined to be *R* and *S* based on total synthesis [35] and X-ray analysis [36]. The *E* geometry of the ethylidene double bond was determined by comparison of the ^{13}C NMR of C-1 and C-3 in *E* and *Z*-4-ethylidene-L-proline diphenylmethyl esters [9] followed by total synthesis of both isomers [35]. It was resulted that the chemical shift of C-1 in *E*-form appear in high field compare to *Z*, while the C-3 was observed in low field. These differences were attributed to the steric shielding effect of the methyl group (C-13).

The seven member diazepandione ring (ring B) of several pyrrolo[1,4]benzodiazepines derivatives is subject to various modifications. These occurred in most cases only at the amide C-11, such as reduction, the presence of imine between N-10-C-11, or in some rare cases the presence of methylene at C-11 as in SEN-215 isolated from *Streptomyces cylindrosporus* S.E.N.-215. SEN-215 has analgesic, sedative, and antispasmodic activity [37]. It is also reported to be synthesised from the conversion of *E* or *Z*-pretomaymycin [38].

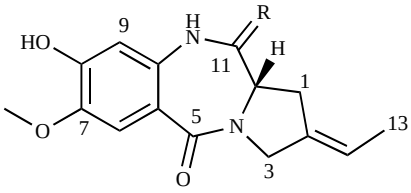
Besides the ethylidene substituent on the five member ring, several PBDs compounds have been isolated with oxygenated substituent such as hydroxy and methoxy group at C-2 or C-3. Examples like neothramycin A, B, RK-1141A and B, chicamycin A and B (originally designated as BBM2040 A and B [39]) have been reported. The latter compounds exhibited antitumor effects on murine leukemia along with weak antimicrobial activity against some Gram-positive bacteria [12]. Compound DC-81, isolated from *Streptomyces roseiscleroticus* DC-81, is among the rare PBDs without substituent at C-2 [40].

Compound YN, which was isolated from the *Streptomyces* sp. YM8-053, is the only compound of this group with a fully unsaturated C-ring (pyrrole). It showed antitumor activity with an IC50 of ca 9.4 μM against human lung cancer A549 [41].

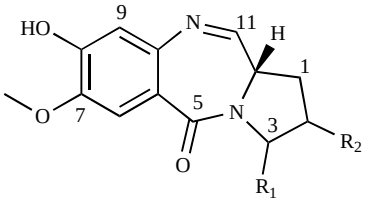
2.4.3. 8-Methoxy-9-hydroxy Substituted Aromatic Ring

Members of this group include the limazepines which are produced by *Micrococcus* sp. a bacterium isolated from the Black Water Ecosystem (BWE) in Indonesian. Limazepines A, B1, B2, C, D and E are structurally related. Limazepines B1 and B2 were isolated as an inseparable diastereoisomeric mixture, which could easily be converted to limazepine C and finally to limazepine D upon prolong staying in $CDCl_3$.

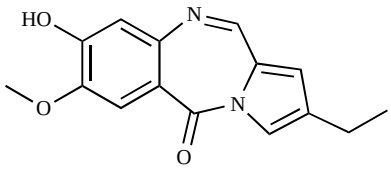
They possess either ethyl of ethylidene group at C-2 of the five member ring. They showed moderate antibacterial and antitumor



Tomaymycin: R = α-OCH_3, H
Oxotomaymycin: R = O
SNE-215: R = H, H
11-demethyltomaymycin: R = OH, H
Tomaymycin I: R = OEt, H



Chicamycin B: R_1 = α-OH; R_2 = H
Neothramycin A: R_1 = H; R_2 = α-OH
Neothramycin B: R_1 = H; R_2 = β-OH
DC-81: R_1 = H; R_2 = H



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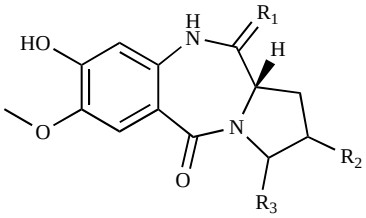


Chicamycin A: R1= α-OCH_3, H; R_2 = α-OH; R_3 = H
RK-1441A: R_1= β-OH, H; R_2 = H; R_3 = α-OCH_3
RK-1441B: R_1= O; R_2 = H; R_3 = α-OH

activities. The structures of limazepines A-F are indicated in Fig. (**1**).

The structure elucidation of limazepines was determined using spectroscopic (1D-, 2D-NMR, MS, IR and UV) methods. The ^{1}H and ^{13}C NMR data are indicated in the supporting information.

2.5. Group of Trisubstituted Aromatic Ring

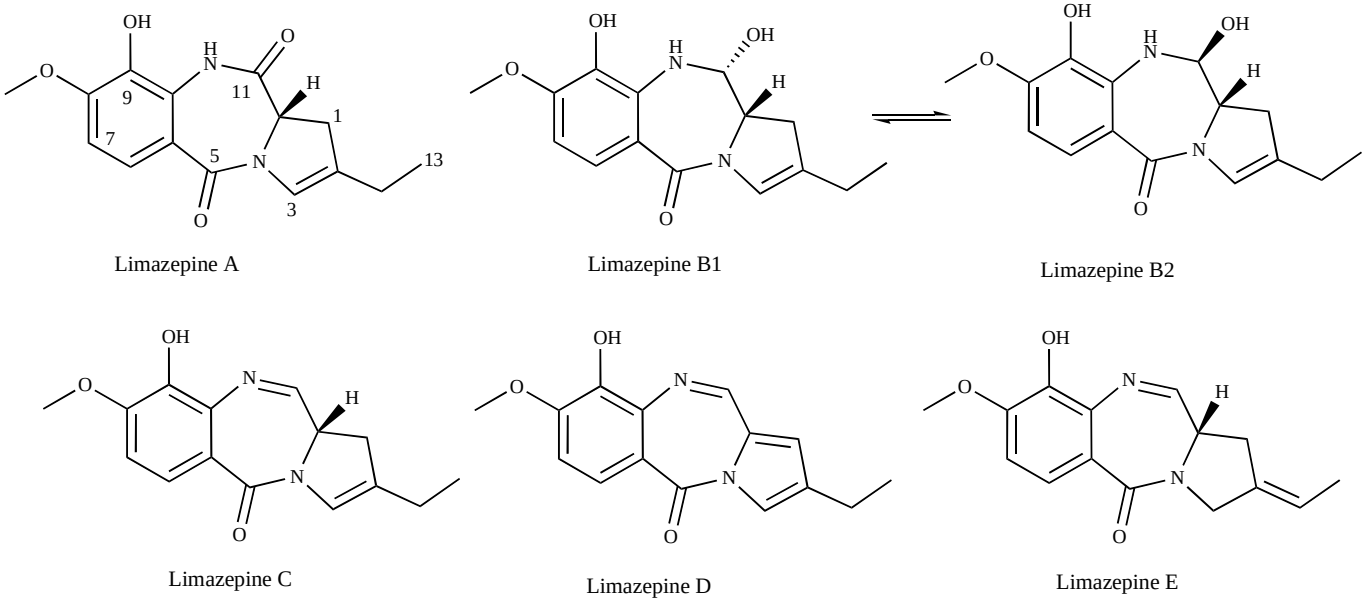
This group contains so far only one member, the glycosylated sibinomycin.

2.5.1. 7-Sibirosamide-8-methyl-9-hydroxy Substituted Aromatic Ring

Sibiromycin isolated from *Streptosporangium sibiricum* [42] is the first reported PBD with an amino sugar. Its chemical structure that differs from all other reported PBDs, and it is the most potent antitumor agent among PBDs [10,14]. To date, in addition to sibiromycin only two glycosylated PBDs have been reported. The structure of sibiromycin was first assigned by chemical degradation and contains a pyrrole C-ring, later the structure was revised based on spectroscopic data followed by synthesis of the aglycone moiety [43].

3. STEREOCHEMISTRY

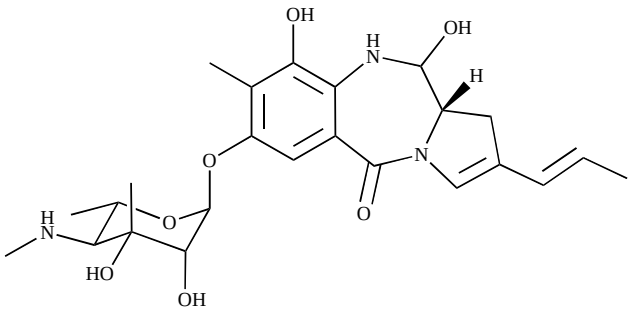
The stereocenters in all natural PBDs are generally at position C-11, C-11a, but substitution at C-2 or C-3 of the C-ring are also observed. There has been no report of natural PBDs with substituents at C-1 or C-5. Most of the compounds reported in the literature, indicated that naturally occurring PBDs possess the same β-



Limazepine A | Limazepine B1 | Limazepine B2

Limazepine C | Limazepine D | Limazepine E

Fig. (1). Structure of limazepines A-F.



Sibiromycin

orientation at C-11a with *S* configuration, whereas substituents at C-11 have an α-orientation with *R* configuration. These observation were based on the interpretation of NMR spectroscopy and support by crystal structure analysis [36]. It should be noticed that so far only four compounds, abbeymycin, limazepine B2, RK-1441A and tilivalline, have been described with a β-oriented stereochemistry at C-11a. Many reports suggested that the lack of COSY coupling between H-11 and H-11a is commonly observed in the anthramycin-tomaymycin group of antibiotics which have 11-*R* and 1la-*S* configurations [1, 12]. This lack of *J*-coupling indicates that the dihedral angle of H(11)-C(11)-C(11a)-H(11a) is approximately 90° [35]. Orientation at C-2 was found as α in chicamycins A and B, but no absolute configuration have so far been reported. Both α and β orientations have been reported for substituted C-3; 3-α for neothramycin A, RK-1441A and RK-1441B and 3-β for neothramycin B [12].

Besides the stereochemistry at C-11, C-11a, C-2 and C-3 of PBDs, the geometry on the double bond C-12-C-13 of the propenamide or C-12-C-13 and C-2-C-12 of the propylidene substitutent at C-2 have all been determined to be *E*, on the basis of their coupling constant in which is in the range of 15-17 Hz [6, 8]. In addition, the ethylidene moiety in tomamycin and prothracarcin have been determined to be *E* by total synthesis [43, 44] of both isomers followed by comparison of their NMR data. In general, it is reported that the chemical shift of C-1 of the *E*-isomer is influenced by the steric shielding effect of the methyl group leading to a shift to the highfield side, as compared to that of the downfield shift of *Z*-isomer. On the other hand, the C-3 signal of the *E*-isomer shifts to the downfield side, as compared to its counterpart *Z*-isomer in the highfield [9].

4. BIOLOGICAL ACTIVITY OF PYRROLOBENZODIAZEPINES

The biological activities of anthramycin, tomaymycin, sibiromycin, neothramycins have been described by Hurley [10] and will not be discussed here. Although the antibiotics of the group pyrrolo[1,4]benzodiazepines are very active antitumor agents, weak antibacterial activity have also been reported [2]. Abbeymycin exhibited weak *in vitro* activity against limited number of anaerobic bacteria such as *Bacteroides fragilis* ATCC 25285, *B. loescheii* ATCC15930, *B. thetaiotaomicron* ATCC29 and *Peptococcus asaccharolyticus* ATCC14963 with MIC ranging from 16-64 μg/ml [25]. Prothracarcin with unsusbtituted aromatic ring indicated weak activity against Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and -negative bacteria (*Escherichia coli, Salmonella typhosa, Shigella sonnei*) with MIC ranging from 50-100 μg/ml. Cytotoxicity assay of prothracarcin indicated its antitumor activity against Murine leukaemia P388 [28].

Chicamycin A and B also showed weak activity against some Gram-positive and fast-acid bacteria, but they do not induce prophage in lysogenic bacteria up to a concentration of 100 μg/ml [45]. These activities were observed for *Streptococcus pyogenes*, *Micrococcus luteus*, *Micrococcus flavus* and *Mycobacterium* strains [45]. The antitumor activity of chicamycins were evaluated against mice, but only chicamycin A showed activity against P388 leukemia and sarcoma 180, while chicamycin B was inactive [45]. The LD_{50} in acute toxicity of chicamycin A and B were found to be 28 mg/kg and 57 mg/kg, respectively [45].

Weak antibacterial activity is also reported for the glycosylated pyrrolo[1,4]benzodiazepines DC-102 against the Gram-positive bacteria *S. aureus, B. subtilis* and *Streptococcus faecium* with MIC of 83, 42, 83 μg/ml, respectively. DC-102 was most effective against murine leukemia P388 showing significant increased life span at a dose of 0.5 mg/kg. The LD_{50} of the DC-102 is 1.5 mg/kg in mouse [30, 31].

A broad antimicrobial spectrum was observed for mazethramycin methyl ether. This compound also has antitumor activity against leukemia L-1210 [6]. Tilivalline has been reported to possess strong cytotoxicity against mouse leukaemia L 1210 [46].

It has been demonstrated that the PBDs exert their antitumoral activity by binding to double stranded DNA, but not to RNA, through a covalent bond. For the mechanism of action, the reaction site has been determined to be N-10-C-11 which can exist in different modes: carbinolamine methyl ether, carbinolamine, imine and the amino-aldehyde. They all are able to alkylate N2-guanine residue [15].

5. CONCLUSION

Although the pyrrolo[1,4]benzodiazepines possess weak antibacterial but potent antitumor activities against various cancer cell lines, no natural PBDs are currently used in the clinic due to their cardiotoxicity as well as tissue necrosis problems [10]. To solve these problems, new PBD analogs have been synthesized and tested. For instance, the pyrrolo[1,4] benzodiazepine SJG-136, a synthetic dimer based on the naturally occurring anthramycin was found to be a highly efficient cross-linking agent of naked DNA. A comparison of SJG-136 results profiles by pattern recognition with that of 60,000 compounds tested in the NCI 60 cell line screen indicated that the agent has an activity pattern similar to some DNA binding agents [47]. However, the activity pattern of SJG-136, a pyrrolobenzodiazepine dimer, differed from three chemically chelated pyrrolobenzodiazepine monomer compounds. In addition, the mean graph activity pattern of SJG-136 did not align with the gene expression cluster patterns associated with any known chemotherapeutic agents, suggesting that SJG-136 possesses a unique mechanism of action [47, 48]. The drug is currently in phase I clinical trials in the US and UK [20].

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