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Graphical Abstract

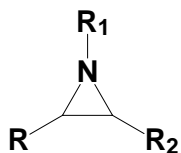
Aziridine alkaloids as potential therapeutic agentsFyaz M. D. Ismail¹, Dmitri O. Levitsky² and Valery M. Dembitsky^{3*}

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Present review describes research on more than 130 natural anticancer, antibacterial agents isolated from terrestrial and marine sources and semi- and synthetic biologically active aziridine alkaloids. These compounds contain the group:



Invited review

Aziridine alkaloids as potential therapeutic agents

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The present review describes research on natural aziridine alkaloids isolated from both terrestrial and marine species, as well as their lipophilic semi-synthetic, and/or synthetic analogs. Over 130 biologically active aziridine-containing compounds demonstrate confirmed pharmacological activity including antitumor, antimicrobial, antibacterial effects. The structures, origin, and biological activities of aziridine alkaloids are reviewed. Consequently this review emphasizes the role of aziridine alkaloids as an important source of drug prototypes and leads for drug discovery.

Keywords: Alkaloids, aziridine, synthesis, anticancer, antibacterial, pharmacological activity, microorganisms, plants, invertebrates

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Introduction

Three-membered heterocycles are highly reactive molecules, in part due to ring strain. As a consequence of their high reactivity, these small heterocycles play an important role in organic chemistry and as intermediates in synthesis of both organic [1] pharmaceutical [2] and natural product intermediates. Among three-membered heterocycles, aziridines constitute a particularly versatile class of molecule, and as discussed in recent book [3], both physical properties and chemical reactions of aziridines have been the subject of numerous theoretical and experimental investigations which have proved invaluable in understanding the mechanism of drug action of pharmaceuticals containing aziridine warheads for instance, the anti-tumor drug FR900482 [4].

Aziridines, the nitrogenous analogues of epoxides, are a group of natural and/or synthetic organic compounds sharing the aziridine functional group which is a three membered heterocycle with one amine group and two methylene groups. Aziridine (ethylene imine, ethylenimine, azacyclopropane,

aziran, binary ethyleneimine, dimethylenimine, or ethyleneimine) has molecular structure $\text{HN}=\text{C}_2\text{H}_4$. The aziridine possesses bond angles of 59.7° which compared with cyclopropane and oxirane molecules, are considerably smaller than that found in hydrocarbons (109.47°) [5]. Bonding within this type of compound can be explained by invoking a banana bond model (See Fig. 1). Bond angle changes about a central atom can profoundly modulate the electronic properties of a molecule [6,7]. In cyclic amines (1-azacycloalkanes), two bond hybrids which participate in the C-N bonds must increase their *p* character upon decrease of the C-N-C angle about the amine nitrogen atom. As a consequence, increased *s* character is seen in the hybrid involved in the N-H bond and the nitrogen lone pair [8-10]. This hybridization effect of the nitrogen atom induces a decrease of pyramidalization of the N-substituent resulting in a continuous increase of the basic strength upon ring expansion from three- to six-membered cyclic amines. This structural relationship is a manifestation of the Thorpe-Ingold effect [11], i.e., the smaller the C-N-C angle (α), the larger the pyramidalization angle (β) (Fig. 1).

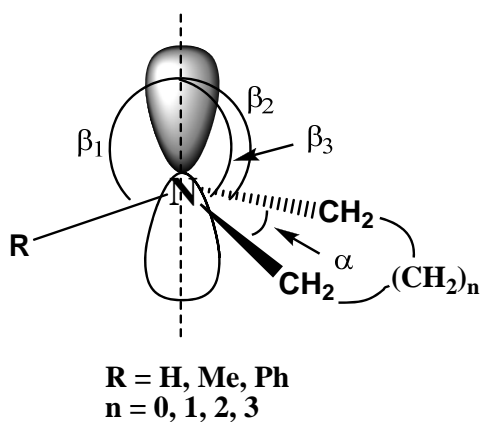


Fig. 1. The view of the C-N-C bond angles in the cyclic amines

The proton affinities, as well as the solution basicities (pK_{BH^+}), of aziridine derivatives are much smaller than those of the corresponding pyrrolidines and piperidines, though the basic strength of azetidines is close to those of pyrrolidines and piperidines. Aziridine is less basic than acyclic aliphatic amines with a pK_a of 7.98 for the conjugate acid due to increased character of the nitrogen free electron pair [12].

The theoretical difference electron density contours in the three-membered ring planes of aziridine (NCC), cyclopropane (CCC) and oxirane (OCC) molecules were calculated by the CNDO/2 MO method, and showed essentially the same bonding electron distributions as the corresponding contours in the more complex molecules mentioned above [13].

The effect of N-methylation on the resonance positions decreases with ring size. N-Alkylaziridines display β and γ effects analogous to acyclic amines; the β effect decreases with branching at the α -carbon.

Ring alkyl groups also induce typical β and γ shifts, and the effect of γ substitution depends on the degree of β -C branching. The influence of ring Me groups on aziridine shifts is additive except for *cis*-2,3- and 1,2-dimethylaziridine in which steric interactions and distortions of molecular geometry probably play a role [14].

While the aziridine group is known as a useful reaction intermediate [1,15], it is also an interesting structural fragment in bioactive compounds. The aziridine's proton accepting properties, its rigidity and its potential reactivity can all contribute to specific molecular interactions with proteins, and indeed several important natural products such as mitomycin C [16], porfiromycin [17], and carzinophilin A [18] contain the aziridine functionality. A number of saccharide derivatives containing the aziridine group have been made, mostly as intermediates [19], but also as glycosidase inhibitors [20].

The toxicity of aziridine derivatives will depend on its own structure and activity whilst sharing the

general characteristics of the aziridine group. As powerful alkylating agents, aziridines have an inherent *in vivo* potency, often based primarily on toxicity rather than specific activity. As an electrophile, substituted aziridines are subject to attack and ring-opening by endogenous nucleophiles such as nitrogenous bases in DNA base pairs, resulting in potential mutagenicity [1,21].

Several groups of rare natural alkaloidal metabolites incorporating the cyclobutane [22], peroxy [23,24], and azetidine moieties [25], and/or their synthetic counterparts possess a broad spectrum of biological activities.

Aziridine alkaloids also belong to a rare and somewhat neglected group of natural products which are known to play a seminal role in the secondary metabolism of some microorganisms, plants and various marine organisms [26]. The aziridine-containing compounds have been of interest as both immuno-modulatory and anticancer agents since the late 1950's [27]. Aziridines are inherently strained making them attractive for study in terms of reactivity and pharmacodynamic action. Ethylenimine (or aziridine, **1**) and some of its simple derivatives, are commercial products in different fields of applied chemistry [28]. Observations of the toxic action of aziridines have prompted extensive investigations involving their synthesis and pharmacological activity, allowing selection and advancement of suitable substances as putative cancer chemotherapeutic agents. Notably a few are enjoying regular clinical use [29]. Bayer strain encourages ring-opening reactions of aziridines in the presence of nucleophiles, imparting useful alkylating properties, despite their powerful mutagenic and toxic activities [30].

Aziridines are highly valuable heterocyclic compounds and are widely used during the synthesis of numerous drugs and biologically active natural products (and their derivatives) [31-36]. Many aziridine alkaloids have anticancer, antibacterial, and/or antimicrobial activity against selected cancer cell lines, pathogenic bacteria, and/or microorganisms strongly indicating that the presence of the aziridine ring in natural as well as synthetic compounds is essential for such activities [37-40].

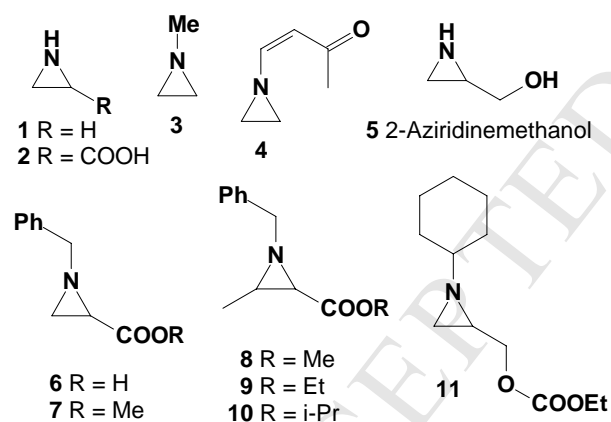
This article reviews natural aziridine alkaloids, with high antitumor, antimicrobial and antibacterial activities and also highlights those semi-synthetic derivatives and analogues which possess therapeutic promise.

Natural aziridine alkaloids

The simple alkaloid, ethylenimine (aziridine, azacyclo-propane, or aziran, **1**) was detected in various foodstuffs including bakers' yeast (*Saccharomyces cerevisiae*) autolyzate [41], in the

volatile flavoring constituents of cooked chicken, beef and pork [42] and beef flavor [43]. Two metabolites (1) and aziridine-2-carboxylic acid (2) were isolated from mushrooms *Agaricus silvaticus* (class Basidiomycetes), both of which have been synthesized [44]. Aziridine-2-carboxylic acid (2) as well as aziridine-containing peptides are vital intermediates in the synthesis of various amino acid and peptide derivatives [45]. Furthermore, (2) and related compounds represent interesting substrates for clarifying enzyme mechanisms, but also as the warhead of novel irreversible proteases inhibitors with a number of potential therapeutic applications [46,47].

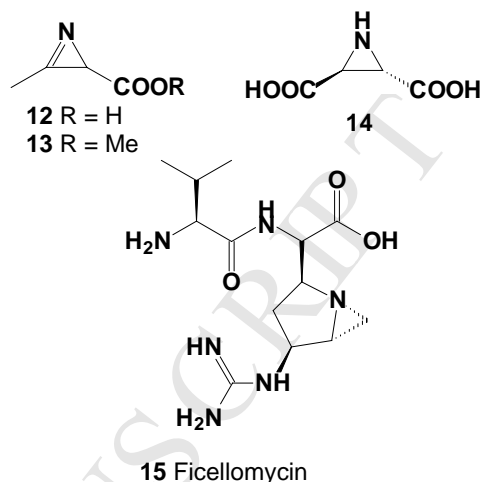
More complex aziridines are found in various plant sources. For instance, 1-methyl-aziridine (3) was detected using GC-MS within onion bulbs (*Allium cepa*, class Liliopsida, order Asparagales, family Alliaceae) [48]. Flue-cured tobacco (*Nicotiana tabacum*, family Solanaceae) contains 4-(1-aziridinyl)-3-buten-2-one (4) [49]. Natural aziridine alkaloids (2,5-11) were detected and isolated from distillate and residue in extractions of dried matter of *Petasites japonicus* (family Asteraceae, Japanese name Fuki) [50] is also known as bog rhubarb or giant butterbur. It is native to Japan, where the spring growth is relished as a vegetable. Consequently its pharmacological properties are of considerable importance.



Since the 1950s, polymerization products of ethylenimine, their polymerizable homologs as well as substitution products were considered useful for disinfecting and preserving textiles, leather, skins, meat, glands, blood, glue, casein (and other albuminous substances), starch, size, dressings, fruits, and vegetables. Their utility in disinfecting floors, walls, stock and portable water vessels, and medical instruments have improved health and safety [51-53].

The azirinomycin (12), 3-methyl-2H-aziridine-2-carboxylic acid, was isolated from a strain of *Streptomyces aureus*. Its methyl ester (13) exhibited broad spectrum antibiotic activity *in vitro* against both Gram-positive and Gram-negative bacteria [54,55]. The carboxylic acid (12) is most active against

Staphylococcus aureus followed by *Proteus vulgaris*, *Bacillus subtilis* and *Streptococcus faecalis*. In contrast, the methyl ester shows its lowest activities against one of the *Staphylococcus aureus* cultures and *Streptococcus faecalis*.



(2*S*,3*S*)-Aziridine-2,3-dicarboxylic acid (also known as *S,S*-2,3-dicarboxyaziridine, 14), which demonstrates antibacterial activity toward *Aeromonas salmonicida*, was isolated from the cultured broth of a *Streptomyces* MD 398-A1 (FERM-P 3217) [56]. The compound (14) was effective against *Pellicularia sasaki* and *Pythium debaryanum* [57]. It is a potent competitive inhibitor of various enzymes including fumarase isolated from pig heart ($K_i = 0.08 \mu\text{M}$) [58], and aspartase of *Escherichia coli* ($K_i = 55 \mu\text{M}$). It also shows antibacterial activity against *Aeromonas salmonicida* [59]. Ethyl esters of aziridine-2,3-dicarboxylic acid inhibited the cysteine proteinase papain [60], whereas peptides containing the aziridine-2,3-dicarboxylic acid building block are inhibitors of several cysteine proteases such as the papain like mammalian proteases [61].

The alkaloidal antibiotic, U-47,929 (also known as ficellomycin, 15) was isolated from *Streptomyces ficellus* [62]. Interestingly, it inhibited the growth of Gram-positive bacteria *in vitro* and is effective in the treatment of experimental *Staphylococcus aureus* infections in mice [63]. Structural elucidation of (15) [64] was eventually achieved by a combination of NMR, mass spectrometry, and formation of derivatives. The 1-azabicyclo[3.1.0] hexane moiety in (15) represents an unusual ring system making ficellomycin a unique natural product [64].

The unique cytotoxic azacyclopropene, *R*-dysidazirine (16) was isolated from the marine sponge *Dysidea fragilis* (Fiji) just over 20 years ago [65]. More recently both the (*Z*) and (*E*) geometrical isomers of *S*-dysidazirine (17a) and (17b) were isolated and were also found to possess cytotoxicity. The dibrominated analogue, *S*-antazirine (18a) and (18b) were also detected within the same marine

sponge *D. fragilis* collected in Pohnpei, Micronesia [66]. Three new ω -halogenated long-chain 2*H*-azirines (**19a,b** and **20**) have recently been isolated from the marine sponge *Dysidea fragilis*, two of them containing a terminal (*Z*)-1-bromo-1-chlorovinyl group, the first such example from a marine invertebrate [67]. Cytotoxic activity of (**17b** and **18b**), and new compounds (**19a,b**, and **20**) is shown in Table 1.

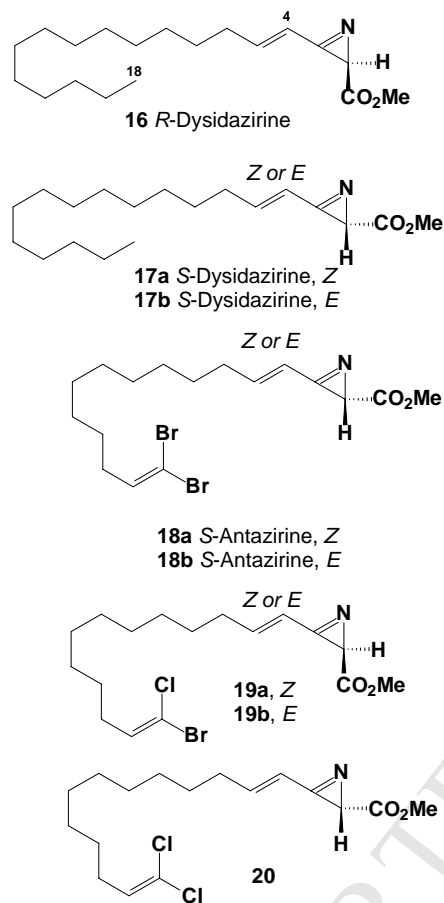
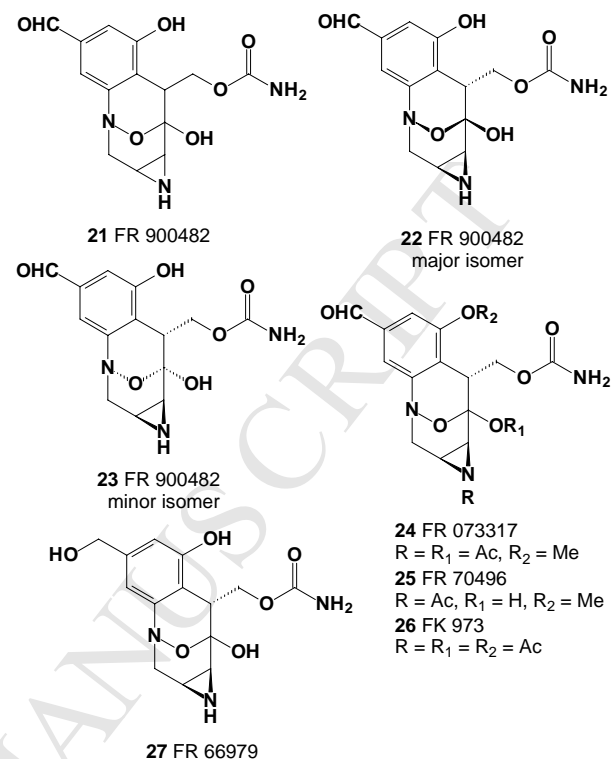


Table 1. *In vitro* cytotoxicity of aziridine-containing fatty acids against HCT-116

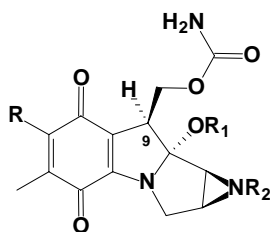
Compound	IC ₅₀ (μg/mL)	IC ₅₀ (μM)
17b	7.9	18.2
18b	8.5	19.6
19a	5.3	13.6
19b	5.9	15.2
20	8.6	24.8

The antitumor antibiotic FR-900482 (**21**) was isolated from *Streptomyces sandaensis* 6897 as a mixture of the two hydroxylamine hemiketal isomers (**22**) and (**23**) [68]. FR-900482 exhibits potent cytotoxic activity against various tumor cells *in vitro*. Furthermore, it possessed a weak antimicrobial activity against some Gram-positive and Gram-negative bacteria [69]. Activity against human LX-1, MX-1, SC-6 and LC-6 tumor cells has been identified

[70]. Quite a number of FR-900482 derivatives were synthesized and some of them showed antileukemic activity [71].

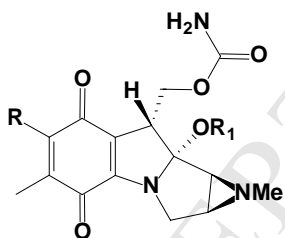


Additionally, FR-900482 inhibited DNA, RNA, and protein reorganization in cell culture of murine L1210 leukemia cells. Whereas FR 900482 did not induce DNA single strand breaks either in the leukemia cells or in plasmid pBR322, it promoted interstrand DNA-DNA cross-links in leukemia cells. An activation of FR 900482 was required prior to induction of interstrand DNA-DNA cross-linking required for cytotoxic action [72]. FK317 (**24**), an analog of FR-900482, had stronger cytotoxic effects against *in vitro* cultured B16, P388, HeLa S3, and KB tumor cell lines. *In vivo* experiments revealed an equivalent antitumor activity of FK317 against P388, M5076, and MX-1, and a more potent antitumor activity against L1210, Colon 38, and LX-1 cell lines as compared with FK973 (**26**) [73]. Both FR900482 (**21**) and FR66979 (**27**) are structurally novel natural products isolated by Fujisawa Pharmaceutical Co. (Japan) in 1987 and have been shown to be highly potent antitumor antibiotics structurally related to the mitomycins [26]. The N-O substructure is bioisosteric with peroxides and the activity of natural products containing this functional group may generate free radicals, especially upon reductive activation. Not surprisingly, studies on the mode of action have established that these new agents form covalent DNA interstrand cross-links both *in vitro* and *in vivo* as a result of the reactive mitosene intermediate generated upon bioreductive activation [for details, see Refs. 74-76].



- 28** Mitomycin A, R = OMe, R₁, R₂ = H
29 Mitomycin F, R = OMe, R₁ = R₂ = Me
30 Mitomycin C, R = NH₂, R₁ = Me, R₂ = H
31 Porfiromycin, R = NH₂, R₁, R₂ = Me
32 9a-Demethylmitomycin A, R = OMe, R₁ = R₂ = H
33 9-epi mitomycin B, R = OMe, R₁ = H, R₂ = Me

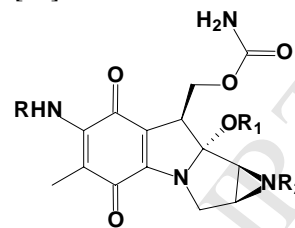
Semisynthetic analogs, such as FK317 (**24**) and FK973 (**26**), were examined for advancement as superior clinic candidates. Although FK317 has been shown to be a potent cytotoxic compound, to date no direct evidence of DNA interstrand cross-link sequence specificity has been reported. In one study, DNA interstrand cross-links were generated by treatment of a synthetic duplex DNA substrate with FK317 and its deacetylated metabolites FR70496 (**25**) and FR157471 [77]. FK973 and all its deacetylated metabolites showed strong cytotoxicity on *in vitro* cultured murine L1210 leukemia cells; however FK973 remained the most potent cytotoxic agent of this series [78]. Synthesis and other biological activities of FR900482 and its analogs have been reviewed [79-81].



- 34** Mitomycin B, R = OMe, R₁ = H
35 Mitomycin J, R = OMe, R₁ = Me
36 Mitomycin D, R = NH₂, R₁ = H
37 Mitomycin E, R = NH₂, R₁ = Me

The mitomycins are potent antibiotics that belong to the family of antitumor quinones. In 1956 mitomycin A (**28**) and B (**34**) were isolated from *Streptomyces caespitosus*, and shortly thereafter mitomycin C (**30**) was discovered within the same strain [82,83]. The *N*-methyl derivative of (**31**), porfiromycin, was isolated in 1960 from *Streptomyces ardens*, which was followed by the discovery of mitomycin from *Streptomyces verticillatus* [84,85]. Among all these different mitomycins, (**31**) enjoyed early widespread clinical use as a consequence of its uniquely superior activity against solid tumors.

Secondly, it possessed reduced toxicity when compared to the natural counterparts (**28**) and (**34**). Mitomycin A, B, and C and porfiromycin also were produced by a *Micromonospora* species KY 11084 [86]. Mitomycins A and C showed antimicrobial activity against *Bacillus subtilis* and *Klebsiella pneumoniae* [87].



- 38** R = R₂ = Me, R₁ = H
39 R = R₁ = Me, R₂ = H
40 R = Et, R₁ = H, R₂ = Me
41 R = Et, R₁ = Me, R₂ = H
42 R = n-Pr, R₁ = H, R₂ = Me
43 R = n-Pr, R₁ = Me, R₂ = H

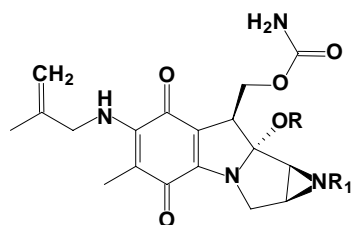
Studies on structural requirements for manifestations of biological activities of mitomycins were especially useful for understanding the role of aziridine moiety. About 70 mitomycin derivatives were synthesized, and it was concluded that the aziridine, quinone, and methylurethan groups apparently were required for full mitomycin antibacterial activity, and some activity remains even after rupture of the aziridine ring [88]. Summarizing available data, the aziridine moiety is markedly more reactive than activated double bonds, e.g. *N*-ethylmaleimide, or halides such as α -iodopropionic acid or chloroacetic acid [89]. The presence of an aziridine ring in mitomycin C suggests that the mechanism of action of the antibiotic is like that of the antitumor alkylating agents. The aziridine ring of mitomycin C interacts with guanine nucleobase of DNA in the alkylation reaction.

An early study showed that mitomycin C had greater activity under hypoxic than aerobic conditions on murine cell lines such as the EMT-6 fibrosarcoma cell line [90]. Mitomycins and porfiromycin, generally nonreactive in the natural oxidized state, behave as bifunctional "alkylating" agents upon chemical or enzymatic reduction, followed by spontaneous loss of the tertiary methoxy (hydroxyl) group and formation of an aromatic indole system. Thus activated, mitomycins and porfiromycin react *in vitro* with purified DNA, linking its complementary strands. A high content of guanine and cytosine favors this cross-linking reaction, which is the basis of the lethal effect *in vivo* of these antibiotics [91].

In a more recent publication [92] it was shown that mitomycin differed considerably from its

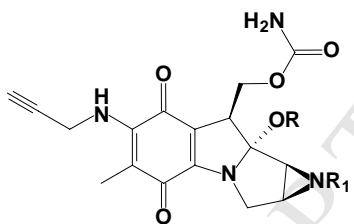
metylated analog in its effects on survival of CHO cells. Under fully aerobic conditions, CHO cells were more affected by mitomycin C than by porfiromycin while under hypoxic conditions the cells became greatly more sensitive to porfiromycin than to mitomycin. The influence of a single and rather trivial substitution in the aziridine moiety on the efficiency of the mitomycins is not yet explained, though a suggestion was made that endogenous enzyme systems may transform these two compounds into other alkylating species in a different way, depending on the intracellular redox conditions [92].

Effects of mitomycin A (1-10 $\mu\text{g/mL}$), mitomycin B (1-50 $\mu\text{g/mL}$), mitomycin C (10-30 $\mu\text{g/mL}$), N-methyl-mitomycin (1-40 $\mu\text{g/mL}$), and porfiromycin (1-60 $\mu\text{g/mL}$) on the *Euglena gracilis* chloroplast system were reported. However, only N-methyl-mitomycin (20-40 $\mu\text{g/mL}$), porfiromycin (40-60 $\mu\text{g/mL}$), and mitomycin B (40-50 $\mu\text{g/mL}$) were effective bleaching agents.



44 R = Me, R₁ = H

45 R = H, R₁ = Me



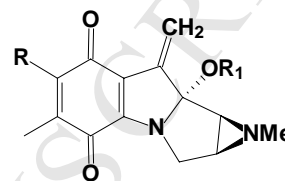
46 R = Me, R₁ = H

47 R = H, R₁ = Me

Thus, only mitomycin derivatives containing an alkyl group on the aziridine nitrogen are effective bleaching agents. The sensitivity of the *Euglena* chloroplast to small structural differences in the active centers of antibiotics demonstrates the usefulness of this organism in finding a relationship between biological activity and chemical structure [93]. Mitomycin A and C were manufactured by fermentation with mitomycin-producing *Streptomyces* and *Micromonospora* or by catalytic isomerization of mitomycin A and mitomycin C, respectively. Both isomers showed antibiotic activities against various bacteria, including *Streptococcus*, *Staphylococcus*, *Bacillus*, *Proteus*, and *Salmonella* [94].

Molecular genetic manipulation of the mitomycin pathway can elucidate the sequence of reactions

involved in mitomycin biosynthesis, as well as provide access to novel mitomycin natural products. Thus, 9a-demethyl mitomycin A (**32**), 9-epi-mitomycin B (**33**), and N-methylmitomycin A (mitomycin F, **29**) have been obtained using mitomycin B as starting material [95]. Mitomycin J (**35**) and mitomycin D (**36**) were isolated as minor antibiotics from *Streptomyces fradiae* SCF5 [96], and mitomycin E (**37**) was obtained from *S. lavendulae* [97]. Mitomycin C, A and F showed anthelmintic activity against gastro-intestinal parasites *Hymenolepis microstoma* and *H. nana* developing in *Tribolium confusum* (Coleoptera, Tenebrionidae) [98].



48 Mitomycin H, R = OMe, R₁ = H

49 Mitomycin G, R = OMe, R₁ = Me

50 Mitomycin K, R = NH₂, R₁ = Me

51 Mitomycin Z, R = NH₂, R₁ = H

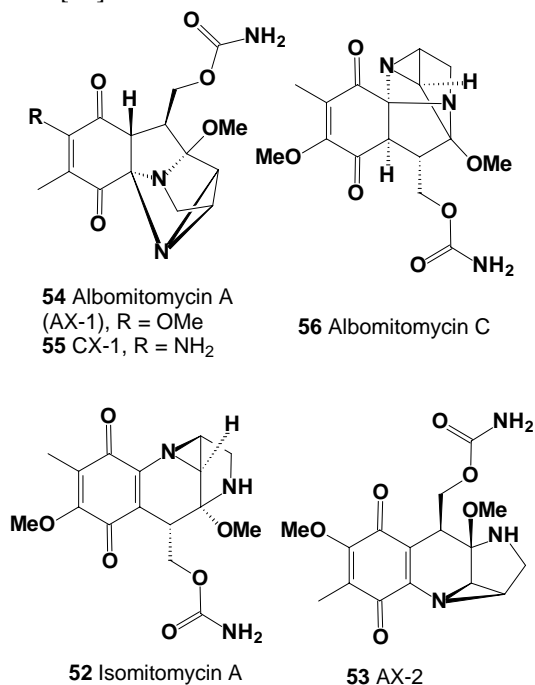
Table 2. Anticancer activity of some mitomycins against Sarcoma 180 cell line*

No.	Δ 9	LD ₅₀	ED ₅₀
28	β	2.1	1.1
29	β	5.0	1.3
30	β	8.4	4.4
31	β	57.0	22.0
32	β	7.5	4.9
34	α	4.5	2.5
35	α	9.0	10.0
48	γ	12.0	6.8
49	γ	130.0	100.0
50	γ	22.0	35.0
51	γ	210.0	82.0

* Substituent at 9 position, a (carbamoyloxy) methyl group with α and β configurations and a vinyl group were taken into account. LD₅₀ and ED₅₀ were used as measures of biological activity. LD₅₀ values of administration of an i.p. route were measured in male ddY mice by probit analysis. ED₅₀ doses that gave 50% inhibition of tumor growth were calculated from the dose-response curve. Sarcoma 180 cells (5 x 10⁶/mouse) were inoculated s.c. into ddY mice on day 0, and drugs were injected i.p. on day 1. Tumor volume was measured on day 7.

Several neoplasm inhibitor analogs (**38-47**) of mitomycin B and C were produced by *Streptomyces caespitosus*. Upon supplementation of the normal fermentation medium for the production of mitomycin C with *S. caespitosus* with a number of primary amines, two new types of mitomycin analogs, described as Type I and Type II, were produced. Type I analogs were related to mitomycin C with the amine substitution at position C7 on the mitosane ring. Type II analogs also contain the same substitutions at C7 but the conformation of the mitosane ring was related to mitomycin B, by possessing an OH at positions C9a and a Me-substituted aziridine [99]. In all cases

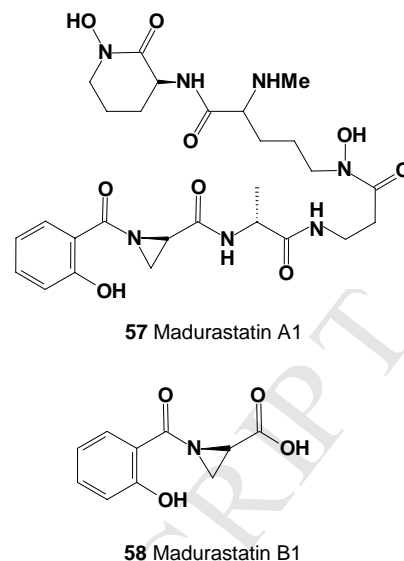
the Type I analogs are more active in a prophage induction test and against L1210 lymphatic leukemia in mice [99].



Mitomycins H (48), G (49), and K (50) were produced by culturing a strain of *S. caespitosus* ATCC 29422 [100]. Mitomycins H, G, K, and Z (51) were also prepared from mitomycin B by cultivating *S. caespitosus* ATCC 27422 [98]. Four isolated antibiotics (48-51) possessed antibacterial activity [101]. Anticancer activity of some mitomycines against Sarcoma 180 cell line is shown in Table 2.

The neoplasm inhibitors, isomitomycin A (52), albomitomycin A (54), and (56) were isolated, together with mitomycin A from *S. caespitosus* culture broth. Both antibiotics were obtained by intramolecular rearrangement of mitomycin A [102]. Anticancer antibiotics AX-2 (53) and CX-1 (55) were isolated from the culture broth of *S. caespitosus*, and obtained from mitomycin C [103]. Other biological activities of different mitomycines, their mechanisms of action, and therapeutic utility have been described in various reviews [26,30,103-109].

A few naturally occurring peptides containing an aziridine ring have been discovered in living organisms. For instance, peptide madurastatin A1 (57), and madurastatin B1 (58), consisting of Ser and salicylic acid moieties, were isolated from the culture broth of a pathogenic *Actinmadura madurae* IFM 0745 strain. Both metabolites showed antibacterial activity against *Micrococcus luteus*, indicating that the presence of the aziridine ring is essential for such activity. Since (57) has a strong affinity with ferric ion attributed to the presence of two hydroxamic acids and a salicylic acid, this low molecular weight chelator is considered a siderophore [110].



Miraziridine A (59) isolated from the marine sponge *Theonella* aff. *mirabilis* unifies within one molecule three structurally privileged elements: (i) (2*R*,3*R*)-aziridine-2,3-dicarboxylic acid, (ii) (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (statine), and (iii) (E)-(*S*)-4-amino-7-guanidino-hept-2-enoic acid (vinyllogous arginine). The alignment of them realized in the tetrapeptide allows for a simultaneous inhibition of the proteolytic activity of trypsin-like serine proteases, papain-like cysteine proteases, and pepsin-like aspartyl proteases. Therefore, this unique compound represents a blueprint for the design of protease class-spanning inhibitors [111,112]. The capability of (59) to inhibit proteases belonging to different classes for trypsin, cathepsin B, cathepsin L, and papain was reported (see Table 3). Miraziridine A [111] also inhibited cathepsin B with an IC₅₀ value of 1.4 μg/mL. Aziridine-2,3-dicarboxylic acid (14) is a rare natural product, reported from a *Streptomyces* [57], and vArg has never before reported as a natural product.

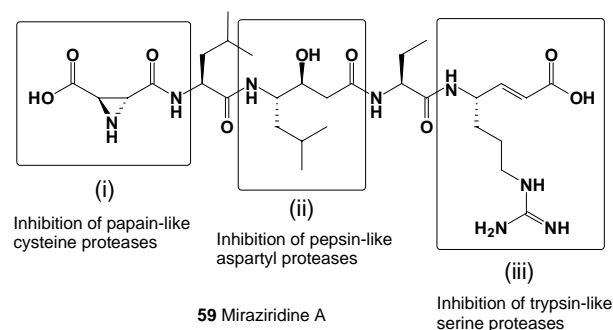
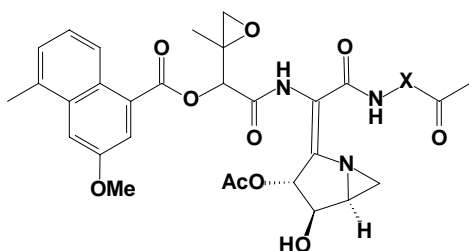


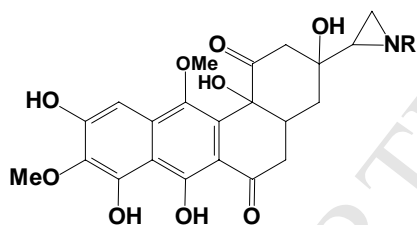
Table 3. Inhibitory properties of Miraziridine A (59)

Protease class	Protease	Affinity
Serine protease	Trypsin	6x10 ⁻⁵ M
Cysteine protease	Cathepsin L	1x10 ⁶ /M/s
	Cathepsin B	1.510 ⁴ /M/s
Aspartyl protease	Pepsin	1.4x10 ⁻⁸ M

Anticancer antibiotics, azinomycin A (**60**) and B (**61**) were isolated from the culture broth of *Streptomyces griseofuscus* S-42227 [113,114]. Azinomycin A and B expressed antitumor activities against P388 leukemia, P815 mastocytoma, B-16 melanoma, Ehrlich carcinoma, Lewis lung carcinoma, and Meth A fibrosarcoma, and it was markedly effective against i.p. inoculated tumors such as P388 leukemia, B-16 melanoma, and Ehrlich carcinoma [115]. Both compounds were active against Gram-positive and Gram-negative bacteria, and L5178Y cells in tissue culture [113]. Azicemicin A (**62**) and B (**63**) were isolated from *Amycolatopsis sulphurea* and its physicochemical properties and antimicrobial activity were defined [116]. It was also isolated from *Amycolatopsis* sp. (MJ126-NF4) cultures, and showed MIC of 50 $\mu\text{g/mL}$ against *Escherichia coli* NIHJ *in vitro* [117,118]. Antimicrobial activities of azicemicin A and B were shown in Tables 4 and 5.



60 Azinomycin A, X = CH₂
61 Azinomycin B, X = C=CHOH



62 Azicemicin A, R = Me
63 Azicemicin B, R = H

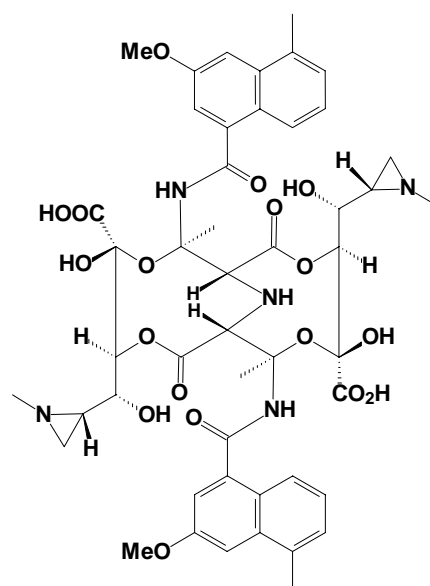
A carboxylic acid antibiotic, carzinophilin, active against Gram-positive bacteria and tumor cells, was isolated from the broth filtrate of *Streptomyces sahachiroi* in 1954 [119,120]. The structure of Carzinophyllin was similar to Azinomycin B, whose partial structure was previously reported [121]. Lown and Hanstock reported the complete structure (**64**) [122]. It has a 2-fold symmetry axis and consists of a dimer of a substituted 1-naphthoic acid attached to a 4-amino-hydroxyvaline linked to an N-methyl-aminohexose moiety. It is the first naturally occurring bis-intercalative (macrocyclic polyoxide) bisalkylator (aziridine) and the mode of its antitumor antibiotic activity is attributed to the reactive moiety (**64**) [122].

Maduropeptin (**65**) is a chromoprotein antitumor antibiotic isolated from the fermentation broth of

Actinomadura madurae [123]. Maduropeptin consists of a 1:1 complex of an acidic, water soluble carrier protein (32 kD) and a 9-membered ring enediyne chromophore possessing potent antibacterial and antitumor properties [123]. It exhibits potent inhibitory activity against Gram-positive bacteria and tumor cells and strong *in vivo* antitumor activity in P388 leukemia and B16 melanoma implanted mice [124]. The biosynthetic gene cluster for the enediyne antitumor antibiotic maduropeptin (MDP) from *Actinomadura madurae* ATCC 39144 was cloned and sequenced. Cloning of the mdp gene cluster was confirmed by heterologous complementation of enediyne polyketide synthase (PKS) mutants from the C-1027 producer *Streptomyces globisporus* and the neocarzinostatin producer *S. carzinostaticus* using the MDP enediyne PKS and associated genes [125].

Table 4. Anti-mycobacterial activities of azicemicins (MIC, $\mu\text{g/mL}$) against the genus *Mycobacterium*

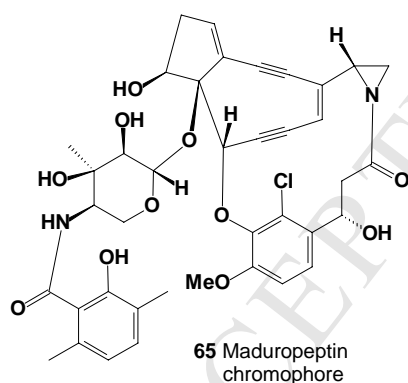
Tested organism	A (62)	B (63)
<i>M. smegmatis</i> ATCC 607	50	12.5
<i>M. vaccae</i> ATCC 15483	50	6.25
<i>M. smegmatis</i> ATCC 607 rifamycin-resistant	50	25
<i>M. smegmatis</i> ATCC 607 paromomycin-resistant		6.25
<i>M. smegmatis</i> ATCC 607 capreomycin-resistant		12.5
<i>M. smegmatis</i> ATCC 607 streptothricin-resistant		25
<i>M. smegmatis</i> ATCC 607 streptomycin-resistant		6.25



64 Carzinophilin A

Table 5. Antibacterial activities of azicemicins A and B (MIC, $\mu\text{g}/\text{mL}$)

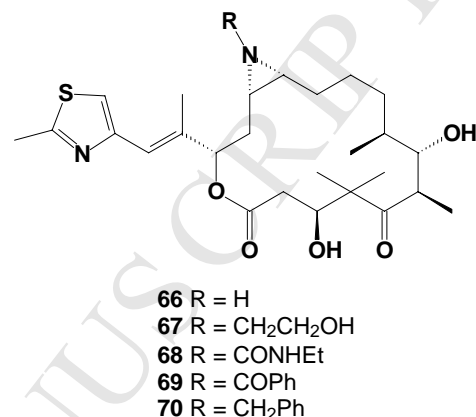
Tested organism	A (62)	B (63)
<i>Bacillus anthracis</i>	100	100
<i>B. cereus</i> ATCC 10702	>100	100
<i>B. subtilis</i> NRRL B-558	>100	100
<i>B. subtilis</i> PCI 219	>100	100
<i>Corynebacterium bovis</i> 1810	25	6.25
<i>Escherichia coli</i> NIHJ	50	25
<i>E. coli</i> K-12	>100	100
<i>E. coli</i> K-12 MLI629	>100	100
<i>E. coli</i> BEM11	100	100
<i>E. coli</i> BE1126	100	100
<i>E. coli</i> BE1186	100	100
<i>Klebsiella pneumoniae</i> PCI602	100	100
<i>Proteus vulgaris</i> OX19	>100	100
<i>P. mirabilis</i> IFM OM-9	>100	100
<i>Providencia rettgeri</i> GN311	>100	100
<i>P. rettgeri</i> GN466	>100	100
<i>Pseudomonas aeruginosa</i> A3	>50	>50
<i>P. aeruginosa</i> GN315	>100	100
<i>Staphylococcus aureus</i> FDA209P	>100	100
<i>S. aureus</i> Smith	>100	100
<i>S. aureus</i> MS9610	>100	100
<i>S. aureus</i> No. 5 (MRSA)	>100	100
<i>S. aureus</i> No. 17 (MRSA)	>100	100
<i>Micrococcus luteus</i> FDA16	50	6.25
<i>M. luteus</i> IFO 3333	12.5	1.56
<i>M. luteus</i> PCI 1001	12.5	1.56
<i>Salmonella typhi</i> T-63	100	100
<i>S. enteritidis</i> 1891	100	>50
<i>Shigella dysenteriae</i> JS1 1910	100	25
<i>S. flexneri</i> 4bJS11811	50	100
<i>S. typhi</i> JS11746	100	100



Selected semi- and synthetic aziridine alkaloids as analogs of natural products

The epothilones are a relatively new class of cytotoxic molecules identified as potential chemotherapeutic drugs which were originally identified as metabolites produced by the myxobacterium *Sorangium cellulosum* and/or *Streptomyces coelicolor* CH999 [126]. These compounds inhibited the growth of a broad range of human cancer cell lines *in vitro* with low nM or sub-nM IC_{50} . A series of 12 α ,13 α -aziridinyl epothilone

derivatives as anticancer agents (66-70), were synthesized in an efficient manner from epothilone A. The final semisynthetic route involved a formal double-inversion of stereochemistry at both the C12 and C13 positions. All aziridine analogs were showed cytotoxicity against cancer cell lines. Thus, (67) had IC_{50} value of 4.3 nM against KB cells. The obtained results indicate that the aziridine moiety is a viable isosteric replacement for the epoxide in the case of epothilones [127].

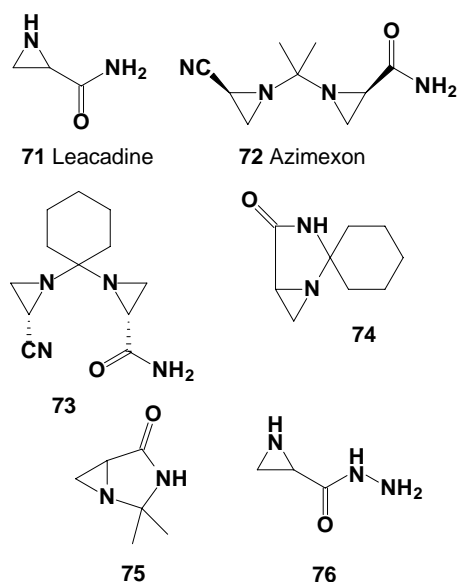


Several derivatives (71-76) of amide aziridine-2-carboxylic acid (also known as leacadine, 71) were prepared as neoplasm inhibitors [128]. Leacadine has been used for treatment of multiple sclerosis [129]. The antitumor efficacy of azimexon (72) in experimental animals and humans was described with respect to its various immunological parameters [130]. Two synthetic aziridine-2-carboxylic acid (2) (71 and 76) showed antitumor activity against a mammary gland tumor in rats [131].

Treatment of (77) with KOH in MeOH at 50 °C resulted in a 60% yield of an isomerization product (putative structures 78,79-81) which in physiological saline solution converts to N-carboxyisoserine. This compound has which had cancerostatic and immunostimulating properties [132].

Imexon (78) is an immunosuppressant which selectively suppresses B-lymphocyte activation and can be used in the treatment of B-cell or plasma cell leukemias or neoplasias. Thus, imexon inhibited the proliferation of stimulated human B-lymphocytes *in vitro* and inhibited the growth of methylcholanthrene-induced fibrosarcoma cells *in vitro*. It was also active against certain autoimmune disorders and infection with Rauscher leukemia virus [133], and also imexon perturbs cellular thiols and induces oxidative stress leading to apoptosis in human myeloma cells (human 8226) [134]. More recently, (5R)- and (5S)- imexons (78a and 78b) have been prepared and used in the treatment of cancer [135]. More details about activity of imexon, and their derivatives, have recently been reviewed [136]. Injection of 10-100 mg BM 06 002 (78) increased immune responses, as indicated by

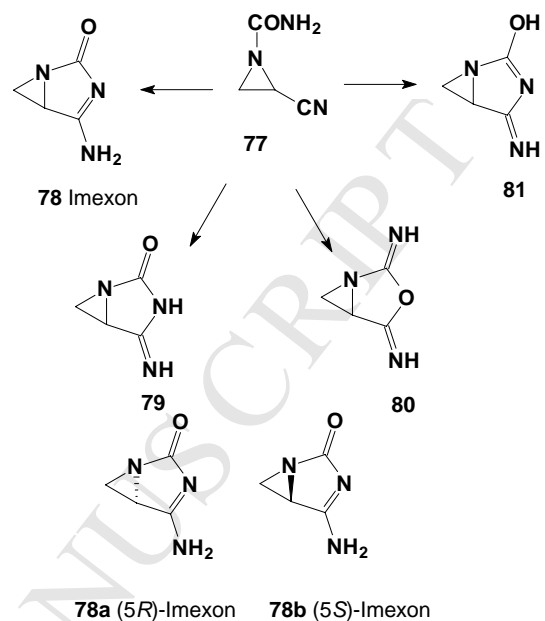
delayed cutaneous hypersensitivity and lymphocyte blastogenesis tests *in vitro*, and also in cancer patients participating in clinical experiments [137].



Two isomeric aziridine-containing analogs of the polyamine spermidine, were synthesized and evaluated for cytotoxic activity against cancer cell lines. Replacement of one of the primary amino groups of spermidine with an aziridinyl functionality yielded either (**82**) or (**83**). N^1 -Aziridinylspermidine (**82**) was cytotoxic *in vitro* against L1210 murine leukemia cells (IC_{50} 0.15 μ M) and HL60 human leukemia cells (IC_{50} 0.11 μ M). N^8 -Aziridinylspermidine (**83**) was slightly less potent against L1210 (IC_{50} 0.31 μ M) and HL60 (IC_{50} 0.30 μ M) cells. Both compounds inhibited incorporation of radiolabeled thymidine, uridine, and valine into trichloroacetic acid-precipitable material by L1210 cells [138].

Neoplasms inhibitor, 3,5-bis(1-aziridinylmethyl)-2,6-dimethyl-pyridine (**84**), was prepared and showed antitumor activity against spindle cell sarcoma 45 and Ehrlich muscle tumor in white rats [139]. Markofane (**85**), a oncostatic agent, was synthesized and its properties and effect on hepatic lipids of rats with sarcoma M-1 were investigated [140]. The body weight of rats with sarcoma M-1 and given a 20% LD_{50} dose of markofane was slightly higher than that of non-treated, sarcomatous rats. Markofane proved quite toxic and a daily dose of 40% LD_{50} resulted in 25% mortality. It exerted insignificant tumor-inhibiting effect on sarcoma M-1 in daily doses of 20 and 40% LD_{50} . Neither sarcoma M-1 nor markofane had any statistical significance on the content of lipids in dry liver. Markofane, 20% LD_{50} , administered to rats with sarcoma M-1, increased the liver content of phosphatides. Preparations of (**86**) were less toxic, had a lower cumulative index, and did not produce profound leukopenia in treated animals and showed

more antitumor activity than known aziridine derivatives. When tested clinically on 80 patients with chronic myeloleukemia, leukocyte counts decreased 30-80% on administration of between 60-80 mg daily doses of A95 [141].



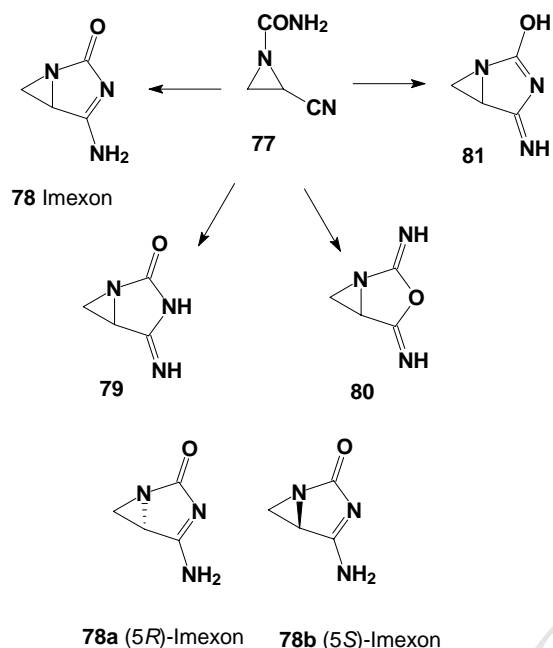
All four prepared compounds (**87-90**) of the paramagnetic urethane phosphoric acid diethyl-enimides inhibited growth of the ascitic form of sarcoma 180 in rats 93-100%, and the three (**87-89**) inhibited Ehrlich ascites tumor growth by 90-98%. Two compounds (**87,88**) inhibited growth of erythromyelosis and Walker carcinosarcoma 100%; while (**89** and **90**) were essentially ineffective. Only compound (**87**) prolonged the survival of animals with leukemia La [142].

Several bioactive phospholipids (**91-96**) have been synthesized. Putative neoplasm inhibitors (**91-94**) showed significant activities in the Walker carcinosarcoma 256 and leukemia L1210 assay systems [143]. The low-melting cytotoxic phospholipids with aziridine groups (**95** and **96**) capable of forming stable dispersions in aquatic glycerol solutions containing 1% egg lecithin were prepared [144].

Fatty acid derivatives (**97-101**) containing an internal aziridine group were prepared by reaction of base with Me iodocarbamates obtained by addition of INCO to a natural fatty acid derivatives followed by treatment with MeOH [145]. Preparation of epimino-stearates (**97**) has also been reported [146].

Synthetic monoglycerides (**102**) with epimino fatty acids showed antimicrobial activity against Gram-positive bacteria and yeasts [147]. Laboratory preparations of 2-ethyl-1-oleoyl-aziridine (**103**) showed a wide spectrum of antifungal and antimicrobial activity [148]. Certain arachidonate aziridines such as 13-(3-pentyl-2-aziridinyl)-5,8,11-

trideca-trienoic acid (**104**) and its methyl ester (**105**) have been synthesized [149] which are inhibitors of arachidonate epoxygenase [150]. Preparation of the fatty acid aziridines (**106-115**) has been described [151]. Bis(aziridine) Me *cis*-9,10;*cis*-12,13-diepiminoctadecanoate, derived from linoleic acid, and tris(aziridine) and Me *cis*-9,10;*cis*-12,13;*cis*-15,16-triepimino-octadecanoate, both derived from linolenic acid, showed cytotoxic and antimicrobial activity as well as remarkable antitumor-promoting and useful neuroprotective effects [152].

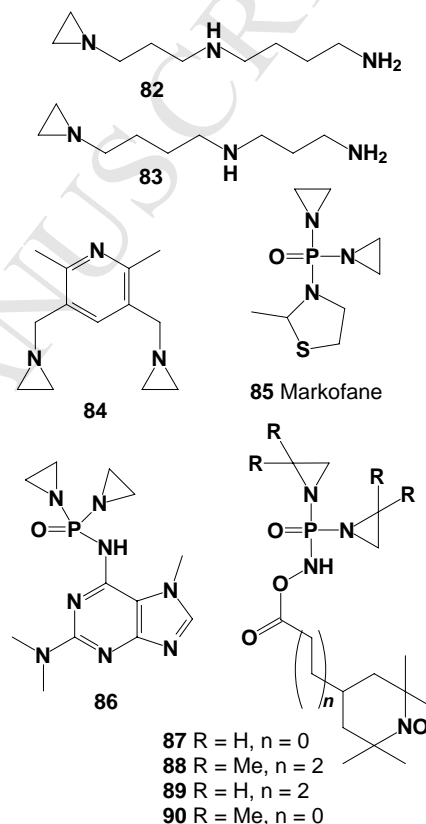


24(*RS*),25-epiminolanosterol (**116**) was a potent noncompetitive inhibitor ($K_i = 3.0$ nM) of the S-adenosyl-L-methionine-C-24 Me transferase from sunflower embryos [153]. Cholesteryl ester of 1-aziridine acetic acid (**117**) showed excellent inhibition of a dimethyl-benzanthrene induced and transplantable mammary adenocarcinoma [154].

Four steroidal alkylating agents (**118-121**) with an aziridine grouping at the C-16 position were synthesized. They were shown anticarcinogenic (oncolytic) activity against implanted mammary carcinoma (milk factor) in C3H/An mice. The steroids 16-(1-aziridinyl)-3 β -hydroxy-pregn-5-en-20-one (**118**), 16-(1-aziridinyl)-3-methoxyestra-1,3,5(10)-trien-17-one (**119**), 16-(1-aziridinylmethyl)-3 β -hydroxy-androst-5-en-17-one acetate (ester) (**120**), and 16-(1-aziridinyl)pregn-4-ene-3,20-dione (**121**), each injected (intraperitoneal, i.p.) at 0.5 mg/mouse/day for 14 days, inhibited tumor growth by 61, 17, 32, and 55%, respectively. None of the compounds were toxic to the host [155].

Potentially cytotoxic estrogen derivatives (**122** and **123**) were prepared [156]. Aziridine derivatives demonstrated a high binding affinity for receptors but

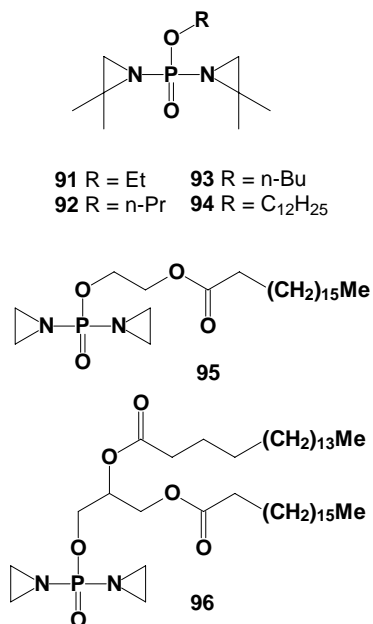
substitution of a bromoacetate group for the aziridine moiety in the same position decreased the binding affinity. Growth of MCF-7 and Evans-T cells from human breast cancer was inhibited by the nitrogen mustards, the mono-nitrogen derivative being the more potent analog. This inhibitory action was unaffected by estradiol or 11 β -chloromethylestra-1,3,5(10)-trien-3,17- β -diol (ORG 4333). Aziridine derivatives either stimulated or inhibited cell growth depending on the concentration. Apparently, the antitumor action of cytotoxic-linked estrogens may be mediated through a mechanism involving estrogen receptors.



Mitomycin C is used extensively to treat various neoplasms, and has led to the discovery of two aminoethylene disulfides, KW-2149 (**124**) and BMS-181174 (**125**). These new compounds differ from mitomycin C only in the C(7) substituent. Novel mechanisms for BMS-181174 and KW-2149 differ from the bioreductive activation pathway commonly accepted for mitomycin C, in that the C(7) aminoethylene disulfide unit undergoes thiol-mediated disulfide exchange to give a mitomycin C thiol derivatives [157].

The cell growth inhibitory activity, antitumor activity and toxicity of M-16 and M-18, the major metabolites of a new mitomycin C (MMC) derivative, KW-2149 (**124**), in both mice and humans were compared with those of KW-2149 and MMC *in vitro* and *in vivo*. The growth inhibitory activity of M-18, a

symmetric disulfide dimer, active against human uterine cervix carcinoma HeLa S3 cells was almost equivalent to that of KW-2149 and their IC_{50} values were about 10-fold smaller than that of MMC. The activity of M-16, a Me sulfide form, was almost equivalent to that of MMC.

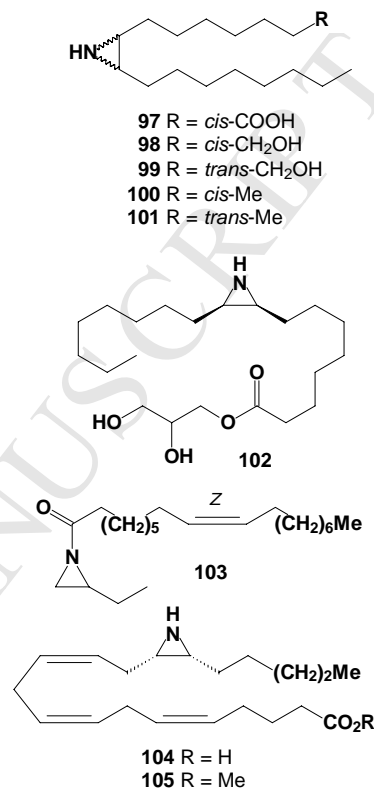


The cell-killing activity of MMC and M-16 was augmented under hypoxic conditions, whereas that of KW-2149 and M-18 was reduced. M-16 also exhibited almost equipotent activities to MMC *in vivo* in terms of various biological parameters, i.e. antitumor activity against murine P388 leukemia, ascitic or solid B16 melanoma or human lung carcinoma xenograft L-27, and bone marrow toxicity in mice.

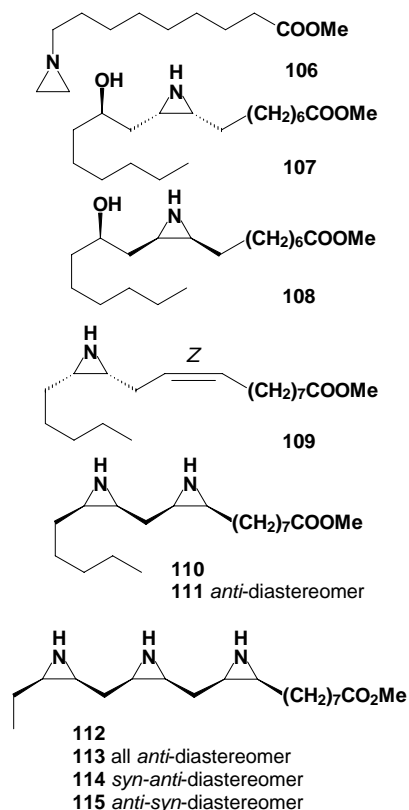
These results *in vitro* and *in vivo* indicate that the antitumor activity and toxicity of KW-2149 might not be mediated by M-16 in mice. On the other hand, M-18 exhibited almost equivalence activities to KW-2149 in this respect, implicating the involvement of M-18 in the biological activities of KW-2149 [158]. Introducing the mercaptoethyl group at the N-7 position of mitomycin C led to the formation of N7,N7'-dithio-diethylene-dimitomycin C (**126**). It showed excellent antitumor activity against sarcoma 180 and leukemia P388 in mice. Amongst the various synthetic compounds, the water soluble conjugate with Et γ -L-glutamyl-L-cysteinyl-glycinate side chain was far more effective against sarcoma 180 and leukemia P388 than mitomycin C [159].

The three dimers (**127**, **128**, and **129**) of mitomycin C (MC), of the aforementioned natural antibiotic and cancer chemotherapeutic agent, were synthesized in which two MC molecules were linked with $-(CH_2)_4-$, $-(CH_2)_{12}-$, and $-(CH_2)_3N(CH_3)(CH_2)_3-$ tethers, respectively [160]. The dimeric mitomycins were designed to react as polyfunctional DNA

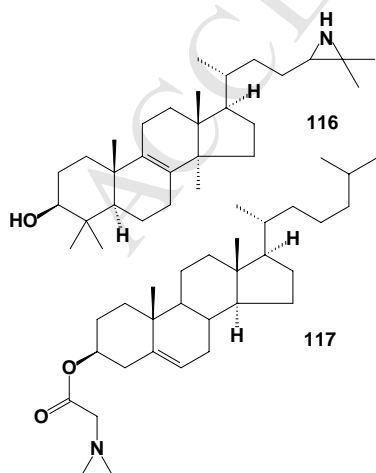
alkylators, generating novel types of DNA damage. To test this design strategy, their *in vitro* DNA alkylating and interstrand crosslinking (ICL) activities were studied using MC, which is itself an ICL agent. Evidence was presented that (**127-129**) multi-functionally alkylate and cross-link extra-cellular DNA and form DNA ICLs more efficiently than MC.



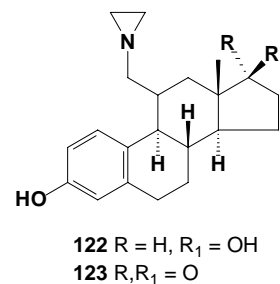
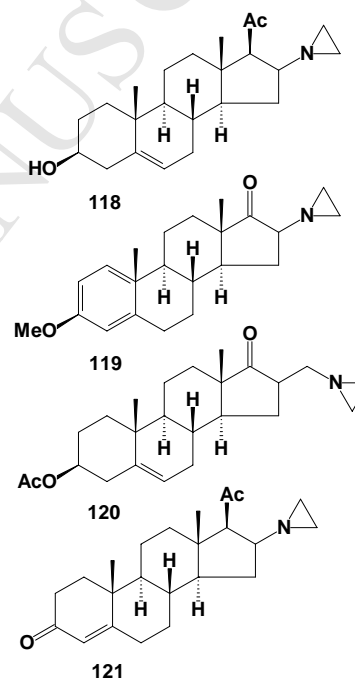
Biological activity depends upon reductive activation which is catalyzed by the same reductases and chemical reductants that activate MC. Dimer **5**, but not MC, cross-linked DNA underwent activation by low pH environments. Sequence specificities of crosslinking of a 162-bp DNA fragment (tyrT DNA) by MC, (**128**), and (**129**) were detected using DPAGE. The dimers and MC cross-linked DNA with the same apparent CpG sequence specificity, but (**129**) exhibited much greater crosslinking efficacy than MC. Greatly enhanced region-selectivity of crosslinking to G-C rich regions by (**129**) relative to MC was observed, for which a mechanism unique to dimeric MCs was proposed. Covalent dG adducts of (**129**) with DNA were isolated and characterized by their UV and mass spectra. Tri- and tetra-functional DNA adducts of (**129**) were also detected. Although the dimers were generally less cytotoxic than MC, dimer (**129**) was highly and uniformly cytotoxic to all 60 human tumor cell cultures of the NCI screen [160]. Its cytotoxicity to EMT6 tumor cells was enhanced under hypoxic conditions. These findings together verify the expected features of the MC dimers and warrant further study of the biological effects of dimer (**129**).

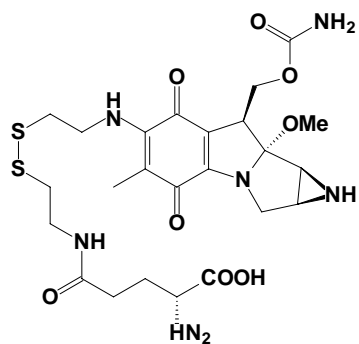


PNU-159548 (4-demethoxy-3'-deamino-3'aziridiny-4'-methylsulfonyl-daunorubicin, **130**), a synthetic derivative of the anticancer idarubicin, has a broad spectrum of antitumor activity both *in vitro* and *in vivo* attributable to its DNA intercalating and alkylating properties [161]. This study was designed to determine the cardiotoxic activity of PNU-159548 relative to doxorubicin in a chronic rat model sensitive to anthracycline-induced cardiomyopathy. PNU-159548 caused a dose-dependent myelotoxicity, with the dose of 0.5 mg/kg per week being equi-myelotoxic to 1.0 mg/kg per week doxorubicin.

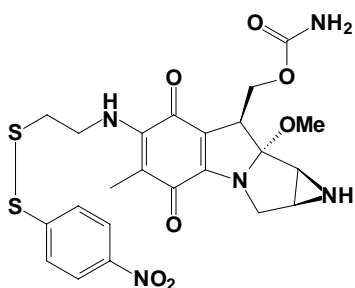


PNU-159548 also caused an increase in liver weight. that was reversible. However, it caused a non-reversible testicular atrophy but, unlike doxorubicin, had no effect on kidney weight. The cytotoxic antitumor derivative, PNU-159548, was significantly less cardiotoxic than doxorubicin at equimyelosuppressive doses. The combination of intercalating and alkylating activities within the same molecule without the cardiotoxic side effects of anthracyclines makes PNU-159548 an excellent candidate for clinic development in oncology. It also showed an $IC_{50} = 2.7$ ng/mL against LoVo colon adenocarcinoma cells [162]. A synthetic preparation of (**131**) showed an IC_{50} of 9.0 μ g/mL against mouse L 5178Y tumor cells, and compound (**132**) had IC_{50} of 0.004 mM against 12 ovarian tumors in the tumor Salmon colony formation test [163].

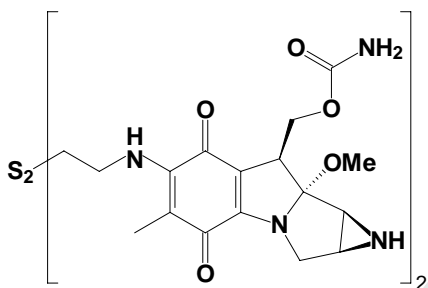




124 KW 2149

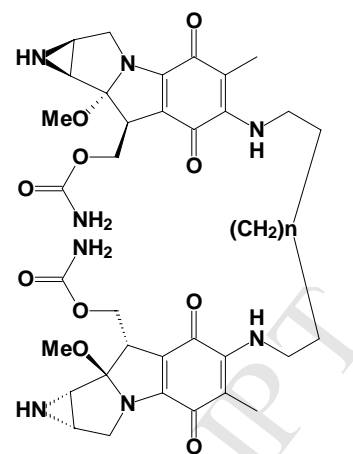


125 BMS 181174



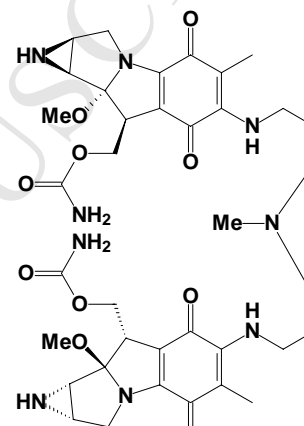
126

Semisynthetic aziridine derivative of colchicine (**133**) have been synthesized by the direct interaction of colchicine with chloroethylamine hydrochloride and also *via* the mono- and diethanolamine derivatives. These compounds had an increased radiomodifying and antitumoral activity and a decreased toxicity compared with the initial colchicine. Results obtained in the National Cancer Institute of the USA from the study of the cytostatic activity of the (**133**) and bis(chloroethyl)amino derivatives on 60 tumor lines were reported [164]. Originally colchicine a soluble alkaloid was extracted from *Colchicum autumnale* also known as autumn crocus, meadow saffron or itkuchala in Uzbekistan which means 'dog poison' [164].



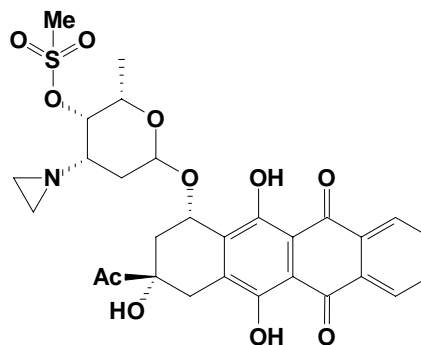
127 n = 0

128 n = 8

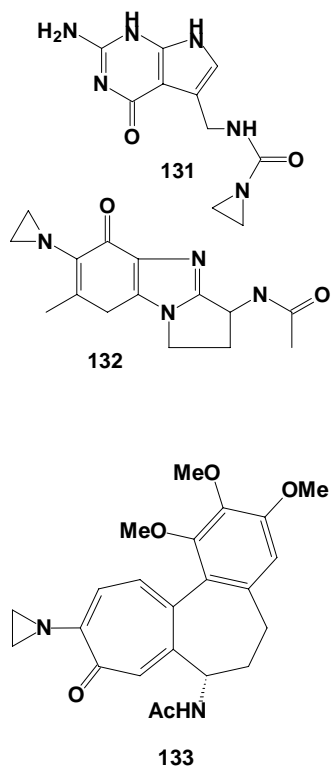


129

Neoplasm inhibitors at C-4 aziridine-bearing paclitaxel (taxol) analogs (**134-136**) were synthesized. The key step in the synthesis is the aziridine ring formation at the C-4 position *via* an intramolecular Mitsunobu reaction [165]. Biological activity of paclitaxel analogs is shown in Table 6.

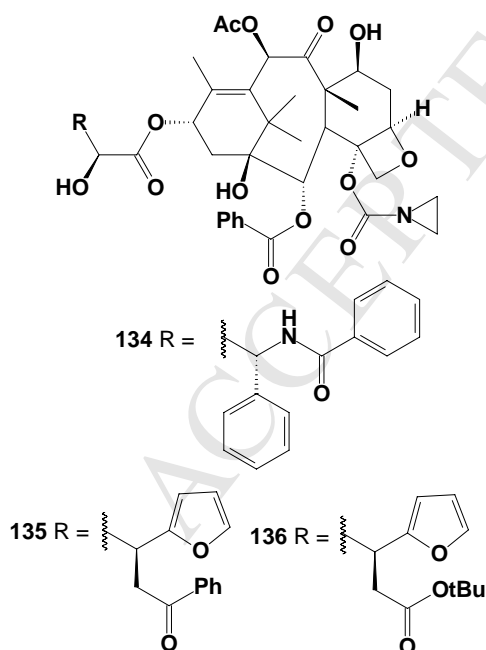


130 Ladirubicin

Table 6. Biological activity of paclitaxel analogs (IC₅₀, nM)

Compound	HCT-116*
134	15.6
135	6.9
136	2.0

*human colon carcinoma



Conclusion

Aziridine alkaloids comprise a rare group of natural products. They are mainly isolated from either microorganisms or plants. They have also been

detected in some marine species. Reported activities for purified alkaloids have shown strong antitumor, antibacterial, antimicrobial, and others activities. A wide spectrum of pharmacological activities is associated with this type of alkaloid which extends to selected synthetic derivatives.

A priori, one should avoid rash conclusions that any of the reported effects of hundreds aziridines are due to their alkylating activity. Quite complex compounds (**21** to **65**) may display additional antioxidant properties; some of them would serve better substances for proteins assuring multidrug drug resistance, such as P-glycoprotein (MDR-1). It is generally accepted that this protein binds its substrates directly from the lipid bilayer rather than from the aqueous cytoplasmic phase. Binding sites of this protein Thus in cancer cells characterized by increased expression of the gene *mdr-1*, these hydrophobic compounds would be more readily exported than such hydrophilic molecules as **1** to **20**.

Natural and/or synthesized aziridine-containing compounds, lipids, steroids, amino acids, as well as their peptide derivatives, have shown to be promising candidates for the development of new drugs toward several diseases, especially neoplasms. No doubt incorporation of an aziridine warhead will allow development of interesting new synthetic and semi-synthetic compounds with clinical utility.

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