# Epipolythiodioxopiperazines from Fungi: Chemistry and Bioactivities

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**Abstract:** Epipolythiodioxopiperazines (ETPs), characterized by a unique bridged disulfide or polysulfide dioxopiperazine six-membered ring, occur in many fungi. Due to its broad spectra of bioactivities, ETPs have drawn wide attention in recent years. This review covers diverse natural sources that produce ETPs, the synthetic chemistry of ETPs and an overview of promising bioactivities exhibited by some well studied ETPs. The plausible biosynthetic hypotheses of ETPs and some new results on antitumor activity of ETPs are also reviewed.

**Keywords:** Epipolythiodioxopiperazines, fungus, bioactivities, biosynthesis, antitumor.

## 1. INTRODUCTION

Epipolythiodioxopiperazines (ETPs), a group of important secondary metabolites from fungi, characterized by the bridged disulfide or polysulfide dioxopiperazine six-membered ring, have been found to have wide range of bioactivities, such as antitumor, antimicrobial, antiviral immunosuppressive and enzyme inhibitory activities. It has been well recognized that disulfide or polysulfide functionalities are the key moiety for their bioactivities, and removal/cleavage of the sulfur bridge or addition of the reducing agent, e.g. dithiothreitol, usually will lead to complete loss of bioactivities. Gliotoxin (**1**), the first reported ETP, was originally isolated from *Gliocladium fimbriatum* in 1932 [1]. The chemistry of 35 ETPs up to the year 1976 had been reviewed by Taylor and Leigh [2]. With the rapid development of new techniques for structural identification and bioactivity screening, ETPs have attracted more intensive attention, and an updated overview of recent progress on ETPs appears necessary. This review covers the source, chemistry and bioactivities of reported ETPs, and SAR studies of their analogues. All ETPs are classified into eight groups according to their structural characteristics. The biosynthetic hypotheses of ETPs and some new results on antitumor activities of ETPs are summarized as well.

## 2. EPIPOLYTHIOPIPERAZINES

### 2.1. Gliotoxins and Analogues

Gliotoxin (**1**), the first reported ETP, was originally isolated from *Gliocladium fimbriatum* in 1932 [1] and lately isolated from *Thermascus* [3], *Candida* [4], *Penicillum terlikowskii* and *Aspergillus fumigatus* [5]. The structure of **1** was proposed by Woodward *et al.* in 1958 without specification of configurational detail [6]. Its absolute configuration was later unambiguously confirmed by X-ray crystallographic analysis in 1966 [7] and its first total synthesis using a novel solvent-dependent Michael reaction as a key step was achieved by Fukuyama *et al.* [8]. Due to its antibiotic, antiviral, immunosuppressive, platelet aggregation inhibitory and antitumor activities [9-14], to date, **1** has been being one of the most studied ETPs in pharmacology. The mechanism of toxicity of **1** was reviewed by Waring and Gardiner, respectively [15, 16]. Gliotoxin-E (**2**), characterized by a same framework of **1** containing a trithio bridge between C-3 and C-10a, was isolated from *P. terlikowskii*, *A. fumigatus* and *T. crustaceus*. Compounds **1** and **2** both were found to have $ED_{50}$ values of 20 ± 10 ng/ml in the macrophage adherence assay [17]. The absolute configuration of **2** was determined by comparison of its CD profile with that of **1** and by chemical convertion to **1** in warm absolute ethanol in about 20% yield. Gliotoxin-G (**3**), characterized by containing a tetrathiodioxopiperazine moiety, was isolated from *A. fumigatus*. Compound **3** exhibited immunosuppressive activity in the macrophage adherence assay, in contrast to its inactive dithiomethylether analogues [18]. The absolute configuration of **3** was determined by comparison of its ORD curve with that of **1** [18], and its partial synthesis was completed by Kirby *et al.* [19]. Dehydrogliotoxin (**4**), isolated from *P. terlikowskii*, was shown to inhibit the growth of *Bacillus subtilis* at similar concentrations of **1** [20] and its first total synthesis was achieved by Kishi *et al.* [21]. Gliontrin A (**5**), a unique ETP containing a nitro-substituted aromatic ring, was obtained from competitive interaction between *Sphingomonas* bacterial strain and *A. fumgatus* fungual strain, and its absolute configuration was assigned by comparison of its CD profile with that of **1**, and was further confirmed by X-ray crystallographic examination. Compound **5** displayed promising antibacterial activity against three strains of MRSA with significant MIC values of 0.78 μg/ml. It also shows potent cytotoxic activity against HCT-116, A549, AGS, DU145, MCF-7 and HepG2 cancer cell lines with $IC_{50}$ values ranging from 0.24 to 2.3 μM in *in vitro* MTT assay [22]. It is worth to note that **5** could not be detected in monoculture broths. This result demonstrated that microbial co-culture could produce novel biologically relevant molecules (compounds **1**-**5** are shown in Table **1**).

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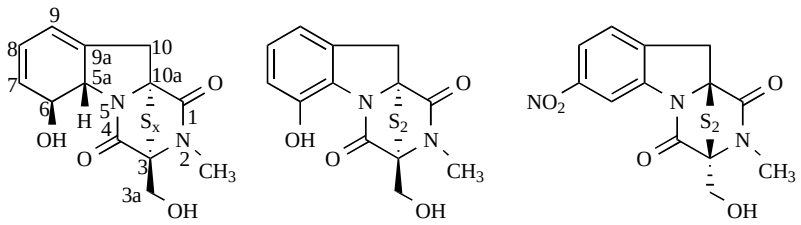
**Table 1. Structures of 1-5**



**1-3** **4** **5**

| No. | Name | x | Species | Refs. |
|---|---|---|---|---|
| **1** | Gliotoxin | 2 | *G. fimbriatum*, *T. sp.*, *C. sp.*, *P. terlikowskii*, *A. fumigatus* | [1, 3, 4, 5] |
| **2** | Gliotoxin-E | 3 | *P. terlikowskii*, *A. fumigaus*, *T. crustaceus* | [17] |
| **3** | Gliotoxin-G | 4 | *A. fumigatus* | [18] |
| **4** | Dehydrogliotoxin | | *P. terlikowskii* | [20] |
| **5** | Glionitrin A | | Coculture of *Sphingomonas* bacteria and *A. fumigatus* | [22] |

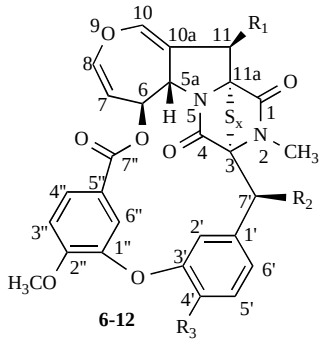
## 2.2. Emestrins and Analogues

Emestrins A (**6**) [23] and B (**7**) [24] were originally isolated from *Emericella striata*. Compound **6**, the first reported example of 15-membered macrocyclic ETP with strong antifungal activity, was formally derived from two molecules of phenylalanine and one molecule of benzoic acid. The structure of **6**, including the absolute configuration, was determined based on a spectroscopic investigation of some derivatives and X-ray crystallography of emestrin methanol solvate. The absolute configuration of **7** was determined by comparison of its CD profile with that of **6**. As determined by the cylinder-agar plate assay, the MIC of **6** against *Gliierella zeae* and *P. expansum* were 10 and 2.5 μg/ml, respectively [23]. Compound **7** showed effective inhibition against *Tricophyton* sp. at 0.78-1.56 μg/ml and *Microsporum* sp. at 3.12-6.25 μg/ml in the *in vitro* assay. Compound **7** also showed inhibitory activity against *Bacillus subtilis* and *Escherichia coli* at concentration of 25 μg/disc [24]. Emestrins C-E (**8-10**), Secoemetrin $C_1$ (**17**) and MPC1001B-H (**11-15**) were produced by fungi from Musk Ox dung and *C.* sp. KY4922, respectively [25, 26]. The absolute configuration of **8** was determined on the basis of NMR and CD profile by comparison with those of **7**, and the conclusion was further supported by the similarity of optical rotation [26]. Compounds **6**, **8-10** and **17** were evaluated for their activity in the CCR2 membrane binding assay using CCR2 receptor CHO cell membrane and $^{125}$I-MCP [25]. Compounds **6**, **8** and **9**, each with two to three sulfur atoms, and with or without a free phenolic group, exhibited ($IC_{50}$ value ~ 0.8 μM) equal activities in CCR2 membrane binding assay. Compound **10**, with four-sulfur atoms, was slightly less active ($IC_{50}$ = 1.1 μM) than **6**, **8** and **9**. Compound **17** exhibited $IC_{50}$ = 9.3 μM and was ten times less active than **6**. In a whole cell assay using human monocyte harvested from Leukopacks, **17** inhibited the $^{125}$I-hMCP-1 binding to CCR2 receptor with $IC_{50}$ value of 7.9 μM, which is about two fold lower than macrocyclic **6** ($IC_{50}$ = 5.4 μM), **8** ($IC_{50}$ = 4.1 μM) and **9** ($IC_{50}$ = 4.2 μM). These results indicate the importance of the macrocycle for inhibitory activity. Compounds **8**, **9**, **11** and **12**, all containing both macrocyclic skeletons and polysulfide bridge, showed antiproliferative activities against DU145 human prostate cancer cell line with $IC_{50}$ values of 9.3, 16, 39 and 12 nM, respectively. However, the decrease of the antiproliferative activieies in **13** ($IC_{50}$ = 83 nM), **14** ($IC_{50}$ = 350 nM) and **15** ($IC_{50}$ = 83 nM) indicates that the macrocyclic ring and polysulfide structures are critical for the antitumor activity [26]. Secoemestrin C (**16**), an epitetrathiodioxopiperazine derivative isolated from an emestrin-producing fungus *E. foveolata*, may be the key intermediate from **6** and/or **7** to **14** and **15** [27] (compounds **6-17** are shown in Table **2**).

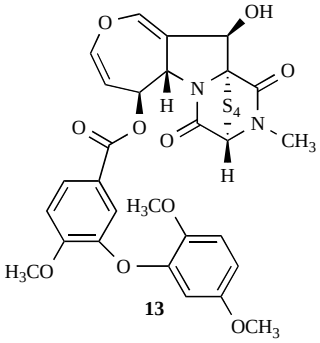
## 2.3. Sporidesmins

Sporidesmins A-J (**18-26,** shown in Table **3**) are toxic metabolites isolated from *Pithomyces chartarum*, a fungus causing farm animals susceptible to a seasonal hepatogenous photosensitization disease known as facial eczema in New Zealand. Successful isolation and structure determination of **18-26** were carried out by Taylor and his coworkers [28-34]. Sporidesmin A (**18**), principal toxin of *Pithomyce chartarum*, was totally synthesized by Kishi *et al.* [35] and its absolute configuration was determined in the early 1960s [7]. Sporidesmin D (**21**) can be obtained by treating **18** with $NaBH_4$ and MeI. The CD profile for the **21** was identical to **18**, indicating no change in absolute configurations [30]. Sporidesmin E (**22**) can be converted to **21** by reduction and methylation, and to **18** when treated with $PPh_3$. When **18** was sulfurated with $P_4S_{10}$ and S, it was possible to isolate **22** from the reaction products [32]. When the tail factions from chromatogram of **22** were re-chromatographed, sporidesmin G (**24**) was obtained. Compound **24** can be converted to **18** on irradiation or treatment with $PPh_3$, and to **21** after reduction and methylation. Similarly, **18** and **22**, when treated with $H_2S_2$, can be converted to **24** [33]. The dimethyl thioether analogues of **18**, sporidesmins D (**21**) and F (**23**), are both inactive metabolites, which indicates that the polysulfide structure is vital for their activity and toxicity

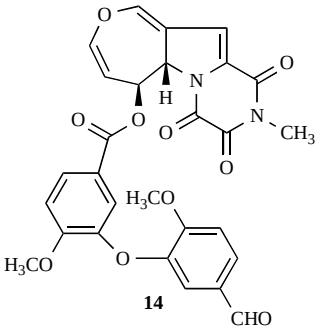
**Table 2. Structures of 6-17**



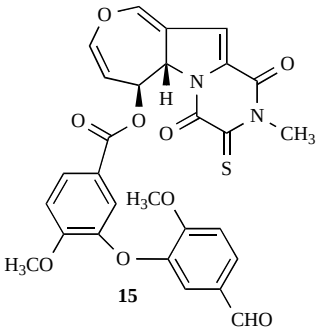
**6-12**



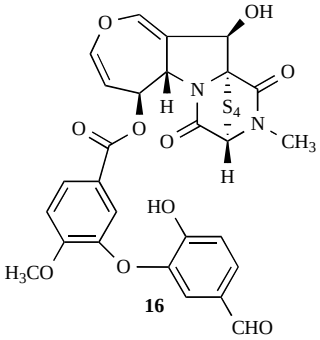
**13**



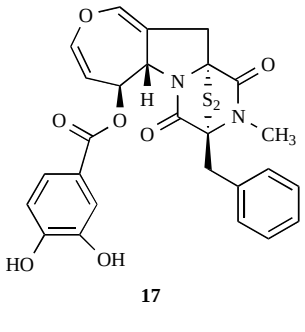
**14**



**15**



**16**



**17**

| No. | Name | $R_1$ | $R_2$ | $R_3$ | x | Species | Refs. |
|---|---|---|---|---|---|---|---|
| **6** | Emestrin | OH | OH | OH | 2 | *E. striata* | [23] |
| **7** | Emestrin B | OH | OH | OH | 3 | *E. striata*, *E. foveolata* | [24, 27] |
| **8** | Emestrin C (MPC1001) | OH | OH | $OCH_3$ | 2 | SM[a], *C. sp.* KY4922 | [25, 26] |
| **9** | Emestrin D (MPC1001D) | OH | OH | $OCH_3$ | 3 | SM, *C. sp.* KY4922 | [25, 26] |
| **10** | Emestrin E | OH | OH | $OCH_3$ | 4 | SM | [25] |
| **11** | MPC1001B | H | OH | $OCH_3$ | 2 | *C. sp.* KY4922 | [26] |
| **12** | MPC1001C | OH | H | $OCH_3$ | 2 | *C. sp.* KY4922 | [26] |
| **13** | MPC1001E | | | | | *C. sp.* KY4922 | [25] |
| **14** | MPC1001G | | | | | *C. sp.* KY4922 | [26] |
| **15** | MPC1001H | | | | | *C. sp.* KY4922 | [26] |
| **16** | Secoemestrin C | | | | | *E. foveolata* | [27] |
| **17** | Secoemestrin $C_1$ | | | | | SM[a] | [25] |

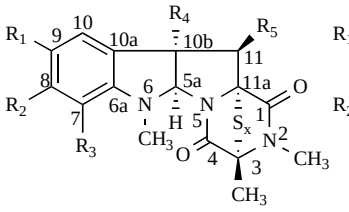
[a]Sterile mycelia.

[30]. Compound **22** was an unstable, but highly cytotoxic metabolite among the above spirodesmins with an end-point of 0.1 ng/ml against HeLa cancer cells [31, 32]. Compound **24** inhibited the growth of HeLa cells at 2 ng/ml, which is being slightly less active than **18** (1 ng/ml) and considerably less toxic than **22**. The toxicity of **24** may be due, in part, to undetected trace of **22** [33].

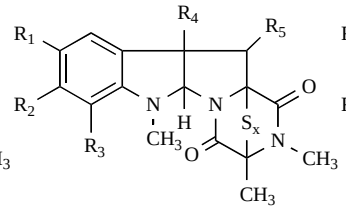
## 2.4. Aranotin and Analogues

Aranotin (**27**), acetylaranotin (LL-S88, **28**) [36] and apoaranotin (**29**) [37] were all produced by a fungus designated as *Arachniotus aureus*. Compound **28** was also isolated from *A. terreus* [38]. The absolute configuration of the key metabolite, **28**, was confirmed by X-ray diffraction analysis [39]. Compound **28** was found to be a viral RNA synthesis inhibitor against strains of rhino-, Coxasckie, polio- and parainfluenza viruses, and to protect mice against lethal infections produced with Coxsackie A21 or influenza S/Md viruses. However, no antiviral activity was observed for LL-S88β, which is a dithiomethyl ether derivative of **28**. The specific action of **28,** completely blocking viral RNA synthesis at levels which are without effect on cellular RNA synthesis, was believed to be the basis for its antiviral

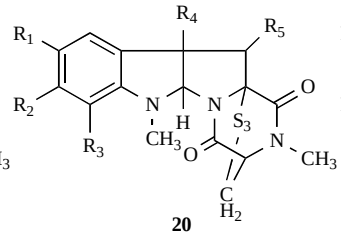
**Table 3. Structures of 18-26**



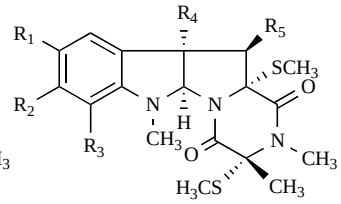
**18, 22, 24**



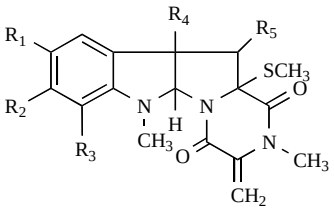
**19, 25, 26**



**20**



**21**



**23**

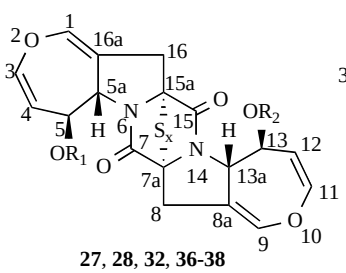
| No. | Name | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | x | Species | Refs. |
|---|---|---|---|---|---|---|---|---|---|
| **18** | Sporidesmin A | Cl | $CH_3O$ | $CH_3O$ | OH | OH | 2 | *Pithomyces. chartarum* | [28] |
| **19** | Sporidesmin B | Cl | $CH_3O$ | $CH_3O$ | OH | H | 2 | *Pithomyces. chartarum* | [28] |
| **20** | Sporidesmin C | Cl | $CH_3O$ | $CH_3O$ | OAc | OAc | | *Pithomyces. chartarum* | [29] |
| **21** | Sporidesmin D | Cl | $CH_3O$ | $CH_3O$ | OH | OH | - | *Pithomyces. chartarum* | [30] |
| **22** | Sporidesmin E | Cl | $CH_3O$ | $CH_3O$ | OH | OH | 3 | *Pithomyces. chartarum* | [31, 32] |
| **23** | Sporidesmin F | Cl | $CH_3O$ | $CH_3O$ | OH | OH | - | *Pithomyces. chartarum* | [30] |
| **24** | Sporidesmin G | Cl | $CH_3O$ | $CH_3O$ | OH | OH | 4 | *Pithomyces. chartarum* | [33] |
| **25** | Sporidesmin H | H | $CH_3O$ | $CH_3O$ | Cl | H | 2 | *Pithomyces. chartarum* | [34] |
| **26** | Sporidesmin J | Cl | $CH_3O$ | H | OH | OH | 2 | *Pithomyces. chartarum* | [34] |

activity [38]. It was also found that **28** inhibited influenza RNA polymerase in the absence of dithiothreitol [40]. Emethallicins A-F (**30-35**) were isolated from *E. heterothallica* and their absolute configurations were established by Kawi and his coworkers [41-44]. Compounds **30-32**, **34** and **35** showed a potent inhibitory activity upon compound 48/80-induced histamine release from mast cell with $IC_{50}$ values of 0.03, 0.08, 1.0, 0.1 and 0.2 μM, respectively, and the $IC_{50}$ values for inhibition of 5-lipoxygenase were determined as 1.7, 1.3 and 2.6 μM for **30-32**, respectively. The inhibitory activity, toward histamine release, of the acetates of **30-32** was usually weaker than those of the original emethallicins, except for emethallicin D monoacetate whose activity was as strong as that of **30**. SCH 64874, SCH 64875 and SCH 64877 (**36-38**), isolated from an unidentified fungus, were found to be potent EGF receptor antagonists, with $IC_{50}$ values of 1.0, 1.0, 1.25 μg/ml, respectively [45] (compounds **27-38** are shown in Table **4**).

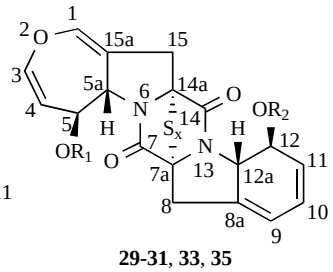
## 2.5. Epicorzines and Analogues

Epicorazines A (**39**) and B (**40**) both exhibiting antibiotic activities were isolated from *Epicoccum nigrum* and *Stereum hirutum* [46-49], and epicorazine C (**41**) was isolated from *Stereum hirutum* [49]. The absolute stereochemistries of **39** and **40** were determined by X-ray analysis and interpretation of their CD and NMR data [47, 48], while the absolute stereochemistry of **41** was derived from NMR data and CD comparison with those of **40** [49]. Moreover, the antibacterial activity assay showed that **39** and **41** were more sensitive to Gram-positive bacteria than Gram-negative bacteria and both compounds also displayed inhibitory effect to *Candida albicans* [49]. Ambewelamides A (**42**) and B (**43**) were isolated from the lichen *Usnea* sp. [50], representing rare members of ETP family characterized by containing ring A/E epoxides and a diketopiperazine epidisulfide bridge in their molecules. Their structures were elucidated *via* a combination of X-ray diffraction and spectroscopic analyses. Compound **42** exhibited potent *in vitro* cytotoxicity (murine leukemia P388: $IC_{50}$ = 8.6 ng/ml) and significant *in vivo* antineoplastic activity (P388: %T/C 140 @ 160 μg/Kg). Rostratins A-D (**44-47**) were isolated and characterized from *Exserohilum rostratum*, and their absolute configurations were determined by the modified Mosher method [51]. Compounds **44-47** showed *in vitro* cytotoxicity against HCT-116 with $IC_{50}$ values of 9, 4.4, 1.6 and 35 μM, respectively. Epicoccins A-H (**48-55**), all possessing common unusual sulfur bridges, were obtained from cultures of a *Cordyceps*-colonizing fungus *E. nigrum*

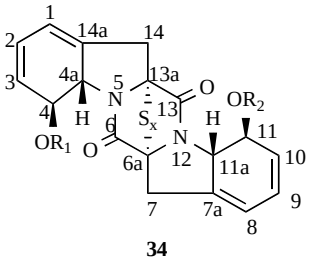
**Table 4. Structures of 27-38**



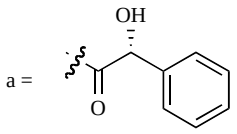
**27**, **28**, **32**, **36-38**



**29-31**, **33**, **35**



**34**



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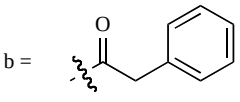

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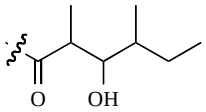


c =

| No. | Name | $R_1$ | $R_2$ | x | Species | Refs. |
|---|---|---|---|---|---|---|
| **27** | Aranotin | H | Ac | 2 | *Arachniotus aureus* | [36] |
| **28** | Acetylaranotin (LL-S88) | Ac | Ac | 2 | *Arachniotus aureus, A. terreus* | [36, 38] |
| **29** | Apoaranotin | H | Ac | 2 | *Arachniotus* | [37] |
| **30** | Emethallicin A | a | b | 2 | *E. heterothallica* | [41] |
| **31** | Emethallicin B | a | b | 4 | *E. heterothallica* | [42] |
| **32** | Emethallicin C | a | a | 4 | *E. heterothallica* | [42] |
| **33** | Emethallicin D | a | b | 3 | *E. heterothallica* | [43] |
| **34** | Emethallicin E | b | b | 2 | *E. heterothallica* | [44] |
| **35** | Emethallicin F | b | b | 2 | *E. heterothallica* | [44] |
| **36** | SCH 64874 | c | c | 2 | NF[a] | [45] |
| **37** | SCH 64875 | c | c | 3 | NF | [45] |
| **38** | SCH 64877 | c | c | 4 | NF | [45] |

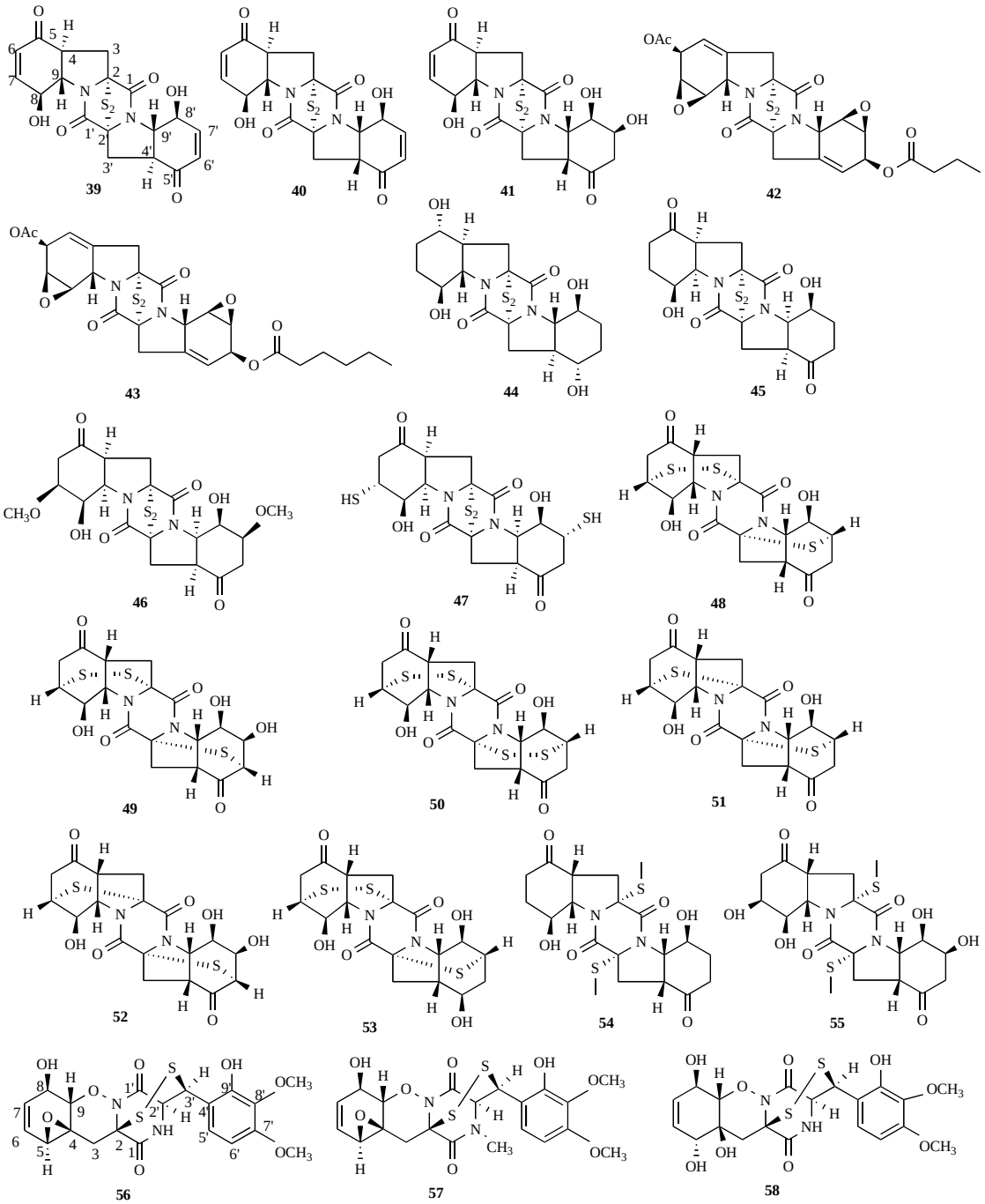
[a]Unidentified fungus.

[52, 53]. The structurally most related known compound **47** could be the biosynthetic precursor for **48-55** [52]. Epicoccin A (**48**) showed modest antimicrobial activity against *Bacilus subtilis*, affording a zone of inhibition of 12 mm at 100 μg/disk, whereas **49-51** were inactive at the same level (ciproflozaxin: 28 mm zone of inhibition at 100 μg/disk) [52]. Compounds **52-55** were tested for *in vitro* anti-HIV-1 activity. However, only the dithiomethyl ethers, **54** and **55**, showed inhibitory effects on HIV-1 replication in C8166 cells with $EC_{50}$ values of 13.5 and 42.2 μM, respectively [53]. Gliovirin (**56**), isolated from *G. virens*, is possibly derived from two molecules of phenylalanine. Compound **56** showed antibiotic activity against *Pyhtium ultimum* and selective activity against members of the Oomycetes [54]. The *N*-methlygliovirin, FA-2097 (**57**), isolated and characterized from *Eupenicillium abidjanum* IFO 8939, exhibited highly antibiotic activity against anaerobic bacteria, especially *Fusobacterium* sp. and *Bacteroides* sp. [55, 56]. Pertrichodermamide A (**58**), isolated from *Trichoderma* sp. BCC 5926, exhibited inhibitory activity against *Mycobacterium tuberculosis* $H_{37}Ra$ with a MIC value of 12.5 μg/ml, and its absolute configuration was established by X-ray crystallographic analysis [57] (compounds **39-58** are shown in Table **5**).

### 2.6. Chaetocins, Verticillins, and Analogues

The ETPs **59**, **61**–**67** were isolated from *Chaetomium* sp. [58, 60-63], and their structures including absolute configuration were determined by chemical, spectroscopic or X-ray analyses, and 11,11′-dihydroxychaetocin (**60**) was originally isolated from the fungus *Verticillium tenerum* [59]. Chaetocin (**63**) was a compound with antibacterial and cytostatic activity [58], and its asymmetric total synthesis was achieved by Sodeoka *et al.* [64]. The bioassay against the growth of Hela cells showed that **59-63** were all cytotoxic with $IC_{50}$ values ranging from 0.02 to 0.04 μg/ml, much stronger than three derivatives of **59** including monosulfide, dethiomethylthio, dethioacetylthio, and the similar SAR were observed in the antibacterial bioassay against *S. aureus* [60]. Compounds **64** and **65**, processing ETP moieties, both displayed comparatively high immunosuppressive activity against Con A-induced (T-cells) and LPS-induced (B-cells) proliferations of mouse splenic

**Table 5. Structures of 39-58**



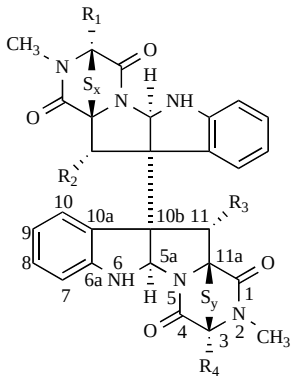
| No. | Name | Species | Refs. |
|---|---|---|---|
| **39** | Epicorazine A | *Epococcum Nigrum, Stereum hirutum* | [46, 49] |
| **40** | Epicorazine B | *Epococcum Nigrum, Stereum hirutum* | [46, 49] |
| **41** | Epicorazine C | *Stereum hirutum* | [49] |
| **42** | Ambewelaminde A | Lichen *usnea sp.* | [50] |
| **43** | Ambewelaminde B | Lichen *usnea sp.* | [50] |
| **44** | Rostratin A | *Exserohilum rostratum* | [51] |

**(Table 5). Contd…..**

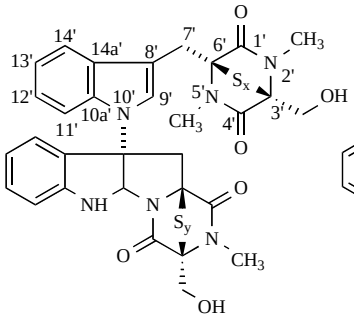
| No. | Name | Species | Refs. |
|---|---|---|---|
| **45** | Rostratin B | *Exserohilum rostratum* | [51] |
| **46** | Rostratin C | *Exserohilum rostratum* | [51] |
| **47** | Rostratin D | *Exserohilum rostratum* | [51] |
| **48** | Epicoccin A | *Epicoccum. Nigrum* | [52] |
| **49** | Epicoccin B | *Epicoccum. Nigrum* | [52] |
| **50** | Epicoccin C | *Epicoccum. Nigrum* | [52] |
| **51** | Epicoccin D | *Epicoccum. Nigrum* | [53] |
| **52** | Epicoccin E | *Epicoccum. Nigrum* | [53] |
| **53** | Epicoccin F | *Epicoccum. Nigrum* | [53] |
| **54** | Epicoccin G | *Epicoccum. Nigrum* | [53] |
| **55** | Epicoccin H | *Epicoccum. Nigrum* | [53] |
| **56** | Gliovirin | *G. virens* | [54] |
| **57** | FA-2097 | *Eupenicillium abidyamum* | [55] |
| **58** | Pertrichodermamide A | *Trichoderma sp.* | [57] |

lymphocytes, while **66** and **67** processing dethio-dimethylthiodioxopiperazine moieties displayed very weak activity in the above mentioned bioassay, indicating the immunosuppressive activity of **64** and **65** might attribute to their ETP moieties in their molecules [63]. This seems to be similar to the result already obtained on other fungal ETPs, sporidesmin A (**18**) and gliotoxin (**1**), by Mullbacher *et al.* [65]. Verticillins A-C (**68-70**), D-F (**71-73**) and G (**74**) were originally isolated from *Verticillium* sp., *G. catenulatum* and *Bionectra byssicola* F120, respectively [66-68]. The absolute configurations of **68** and **69** were elucidated by chemical and physicochemical methods [66]. The stereochemistries for **71-73** were proposed by analogy to other ETPs in this class based on close agreement of relevant $^{1}H$ and $^{13}C$ NMR chemical shifts [67]. Compounds **68-70** exhibited inhibitory activity against Gram-positive bacteria and mycobacteria but no inhibitory activity against Gram-negative bacteria and fungi, and the cytotoxic effects ($ED_{50}$) of **68** and **69** against HeLa cells were both 0.2 μg/ml [66]. Compounds **71-73** showed antibacterial activity at 100 μg/ml, affording inhibitory zone sizes of 23 to 26 mm against *Bacillus subtilis*, and of 11 to 14 mm against *S. aureus* [67]. Compound **74** inhibited the growth of *S. aureus* including MRSA and QRSA with MIC values of 3 to 10 μg/ml [68]. 11,11′-Dideoxyverticillin A (**75**) and 11′-deoxyverticillin A (**76**) were originally isolated from *Penicillium* sp. [69], and **75** was also isolated from *Shiraia bambusicola* [70]. Their absolute configurations were assigned on the basis of NMR and CD experiments. Compounds **75** and **76** were found to exhibit potent *in vitro* cytotoxicity against HCT-116 with $IC_{50}$ values of 30 ng/ml [69], and **75** was found to be a structurally novel antiangiogenesis inhibitor [70]. The concise enantioselective total synthesis of **75** was achieved *via* a strategy inspired by the biosynthetic hypothesis by Movassaghi *et al.* [71]. The strategy should be applicable to other members of ETP family for a much thorough investigation of their synthesis, provides valuable insight into the function of enzymes involved in the biosynthesis of these natural products, and provides an access to obtain analogues which may have more promising pharmacological activity. Compounds **77-84** were isolated and characterized from fungus *G.* sp. [72-74]. The stereochemistries for Sch52900 (**77**) and Sch 52901 (**78**) were assumed to be the same as **68** by a direct comparison of their optical rotations and CD profile [72]. Chu *et al.* found **77** and **78** to be inhibitors of *c-fos* protp-oncogene induction and suggested that ETPs exerted antitumor activity by blocking a signal transduction pathway that was common to and necessary for the induction of at least a subset of immediate early genes involved in cell proliferation [72]. *In vitro* immersion tests showed that **68**, **76-83** exhibited antinematodal activity against *Caenorhabditis elegans* ($ED_{50}$ = 30, 10, 50, 50, 25, 30, 200, 200, 200 μg/ml, respectively) and *Panagrellus redicicus* ($ED_{50}$ = 80, 40, 80, 50, 50, 25, 250, 250, 250 μg/ml, respectively), suggesting that monomeric epipolysulfanydioxopiperzaines (**81-83**), with indole moiety, are less active than the dimeric ones (**68**, **76-80**) [73]. Gliocladicillins A (**85**) and B (**86**), as cancer cell proliferation inhibitors and apoptosis inducers, were identified from *Cordyceps*-colonizing fungus using an ecology-based approach [75]. Bionectines A-C (**87-89**) were isolated from *B. byssicola* F120 [76], and T988 A-C (**90-92**) were isolated from *Tilachlidium* sp. [77]. Compounds **87** and **88** exhibited antibacterial activity against *S. aureus* including MRSA and QRSA with MIC values of 10-30 μg/ml, and the dimeric ETP (**71**) showed stronger antibacterial activity with MIC values of 3-10 μg/ml, while **89** showed no antibacterial activity even at 100 μg/ml [76]. **90-92** all showed potent cytotoxicities against cultured P388 leukemia cells with $ID_{50}$ values of 0.25, 2.18, and 0.56 μM, respectively [77]. The

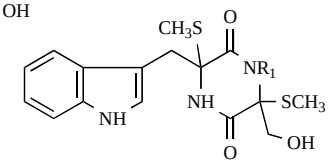
**Table 6. Structures of 59-95**



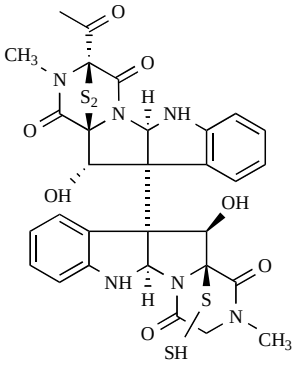
**59-63**, **68-73**, **75-80**, **85**, **86**



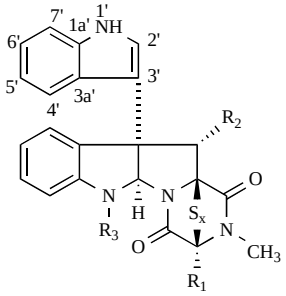
**64**, **65**



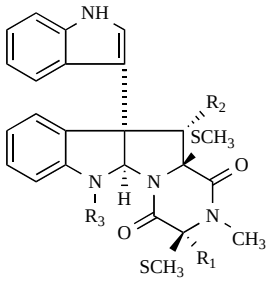
**66**, **67**



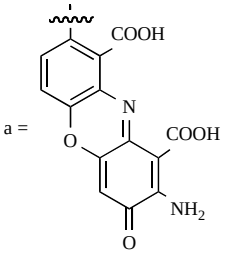
**74**



**81-84**, **87**, **88**, **90**, **92**, **95**



**89**, **91**, **93**, **94**



| No. | Name | $R_1$ | $R_2$ | $R_3$ | $R_4$ | x | y | Species | Refs. |
|---|---|---|---|---|---|---|---|---|---|
| **59** | Chaetocin | $CH_2OH$ | H | H | $CH_2OH$ | 2 | 2 | *C. minutum* | [58] |
| **60** | 11,11′-Dihydroxychaetocin | $CH_2OH$ | OH | OH | $CH_2OH$ | 2 | 2 | *V. tenerum* | [59] |
| **61** | Chaetocin B | $CH_2OH$ | H | H | $CH_2OH$ | 3 | 2 | *C. virescens* var. *thielavioideum* | [60] |
| **62** | Chaetocin C | $CH_2OH$ | H | H | $CH_2OH$ | 3 | 3 | *C. virescens* var. *thielavioideum* | [60] |
| **63** | Chetracin A | $CH_2OH$ | OH | OH | $CH_2OH$ | 4 | 4 | *C. nigricolor*, *C. retardatum.* | [60] |
| **64** | Chetomin | - | - | - | - | 2 | 2 | *C. cochliodes*, *C. globosum*, *C. seminudum* | [61, 62] |
| **65** | Chetoseminudin A | - | - | - | - | 2 | 3 | *C. seminudum* | [63] |
| **66** | Chetoseminudin B | H | - | - | - | - | - | *C. seminudum* | [63] |
| **67** | Chetoseminudin C | $CH_3$ | - | - | - | - | - | *C. seminudum* | [63] |
| **68** | Verticillin A | $CH_3$ | OH | OH | $CH_3$ | 2 | 2 | *V. sp.*, *P. sp.*, *G. sp.*, *G. roseum* | [66,69,72, 73] |
| **69** | Verticillin B | $CH_3$ | OH | OH | $CH_2OH$ | 2 | 2 | *V. sp.* | [66] |
| **70** | Verticillin C | $CH_3$ | OH | OH | $CH_2OH$ | 2(3) | 3(2) | *V. sp.* | [66] |
| **71** | Verticillin D | $CH(OH)CH_3$ | OH | OH | $CH(OH)CH_3$ | 2 | 2 | *G. catenulatum*, *B. byssicola* | [67, 68] |

**(Table 6). Contd…..**

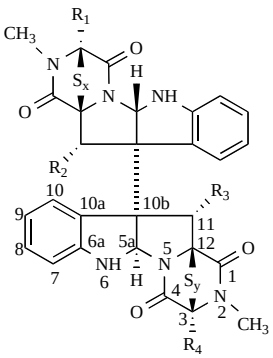
| No. | Name | $R_1$ | $R_2$ | $R_3$ | $R_4$ | x | y | Species | Refs. |
|---|---|---|---|---|---|---|---|---|---|
| **72** | Verticillin E | Ac | OH | OH | Ac | 2 | 2 | *G. catenulatum* | [67] |
| **73** | Verticillin F | $CH(OH)CH_3$ | OH | OH | Ac | 2 | 2 | *G. catenulatum* | [67] |
| **74** | Verticillin G | - | - | - | - | - | - | *B. byssicola* | [68] |
| **75** | 11,11′-Dideoxyverticillin A | $CH_3$ | H | H | $CH_3$ | 2 | 2 | *P. sp.*, *S. bambusicola*, | [69,70] |
| **76** | 11′-Deoxyverticillin A | $CH_3$ | OH | H | $CH_3$ | 2 | 2 | *P. sp.*, *G. roseum* | [69, 73] |
| **77** | Sch 52900 | $CH(OH)CH_3$ | OH | OH | $CH_3$ | 2 | 2 | *G. sp.*, *G. roseum* | [72,73] |
| **78** | Sch 52901 | $CH_2CH_3$ | OH | OH | $CH_3$ | 2 | 2 | *G. sp.*, *G. roseum* | [72,73] |
| **79** | Gliocladine A | $CH_3$ | OH | OH | $CH_3$ | 3 | 2 | *G. roseum* | [73] |
| **80** | Gliocladine B | $CH_3$ | OH | OH | $CH_3$ | 4 | 2 | *G. roseum* | [73] |
| **81** | Gliocladine C | $CH_3$ | OH | H | - | 2 | - | *G. roseum* | [73] |
| **82** | Gliocladine D | $CH_3$ | OH | H | - | 3 | - | *G. roseum* | [73] |
| **83** | Gliocladine E | $CH_3$ | OH | H | - | 4 | - | *G. roseum* | [73] |
| **84** | Glioclatine | $CH_3$ | H | H | - | 2 | - | *G. roseum* | [74] |
| **85** | Gliocladicillin A | $CH_3$ | H | H | $CH(OH)CH_3$ | 2 | 2 | *Cordyceps*-colonizing fungus | [75] |
| **86** | Gliocladicillin B | $CH_3$ | H | H | $CH_2CH_3$ | 2 | 2 | *Cordyceps*-colonizing fungus | [75] |
| **87** | Bionectin A | H | OH | H | - | 2 | - | *B. byssicola* | [76] |
| **88** | Bionectin B | $CH(OH)CH_3$ | OH | H | - | 2 | - | *B. byssicola* | [76] |
| **89** | Bionectin C | H | OH | H | - | - | - | *B. byssicola* | [76] |
| **90** | T988 A | $CH_2OH$ | OH | H | - | 3 | - | *Tilachlidium*. sp, *Plectosphaerella cucumerina* | [77, 78] |
| **91** | T988 B | $CH_2OH$ | OH | H | - | - | - | *Tilachlidium. sp* | [77] |
| **92** | T988 C | $CH_2OH$ | OH | H | - | 2 | - | *Tilachlidium. sp* | [77] |
| **93** | Plectosphaeroic acid A | $CH_2OH$ | OH | a | - | - | - | *Plectosphaerella cucumerina* | [78] |
| **94** | Plectosphaeroic acid B | $CH_3$ | OH | a | - | - | - | *Plectosphaerella cucumerina* | [78] |
| **95** | Plectosphaeroic acid C | $CH_2OH$ | OH | a | - | 3 | - | *Plectosphaerella cucumerina* | [78] |

results indicated that the cytotoxic and antibacterial activities of the ETP class of compounds were mediated in part due to the sulfide bridge in the ETP moiety, while the number of sulfur atoms in the bridge may not significant influence the activity. Plectosphaeroic acids A-C (**92-95**) were all isolated from the fungus *Plectosphaerella cucumerina* together with **90** [78]. Notablely, **93-95** all could inhibite purified recombinant human IDO, a promising molecular target for treating cancer, with identical $IC_{50}$ values of ≈ 0.2 μM, while **90** was completely inactive. Meanwhile, the synthetic cinnabarinic acid was also found to be active in this assay with an $IC_{50}$ value of ≈ 0.2 μM, indicating that some portion of the phenoxazineone fragment may play a key role for inhibitory effect to IDO (compounds **59-95** are shown in Table **6**).

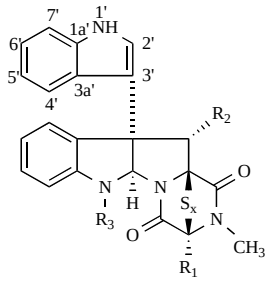
### 2.7. Leptosins

Leptosins A-S (**96-119**), antitumor metabolites from *Leptoshaeria* sp., were discovered by Numata and his coworkers [79-84]. Compound **96** represented the first dimeric ETP with a different configuration at C-5′a and C-10′b respect to other related compounds, such as chaetoins

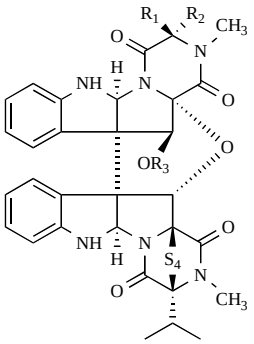
**Table 7. Structures of 96-119**



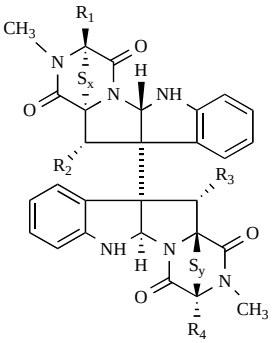
**96-98, 102-105**



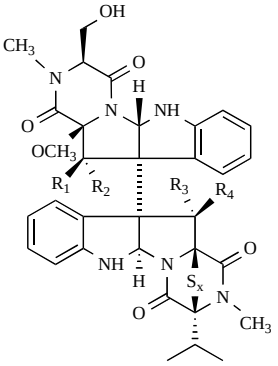
**99-101**



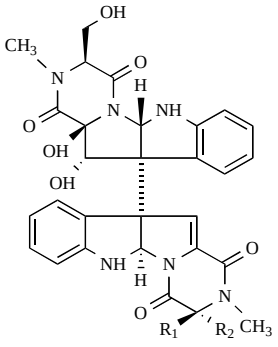
**106, 107**



**108-110**



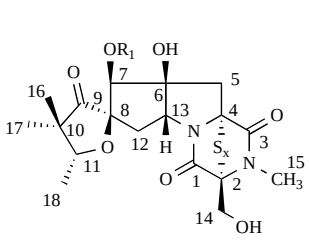
**111-114**



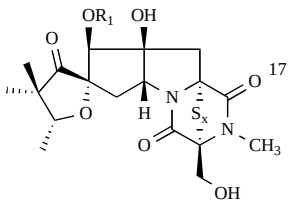
**115-119**

| No. | Name | $R_1$ | $R_2$ | $R_3$ | $R_4$ | x | y | $ED_{50}$ | Species | Refs. |
|---|---|---|---|---|---|---|---|---|---|---|
| **96** | Leptosin A | $CH_2OH$ | OH | OH | $CH(CH_3)_2$ | 4 | 2 | 1.85 ng/ml | *L. sp.* | [79] |
| **97** | Leptosin B | $CH_2OH$ | OH | OH | $CH(CH_3)_2$ | 3 | 2 | 2.4 ng/ml | *L. sp.* | [79] |
| **98** | Leptosin C | $CH_2OH$ | OH | OH | $CH(CH_3)_2$ | 2 | 2 | 1.75 ng/ml | *L. sp.* | [79] |
| **99** | Leptosin D | $CH(CH_3)_2$ | OH | H | - | 2 | - | 86 ng/ml | *L. sp.* | [79] |
| **100** | Leptosin E | $CH(CH_3)_2$ | OH | H | - | 3 | - | 46 ng/ml | *L. sp.* | [79] |
| **101** | Leptosin F | $CH(CH_3)_2$ | OH | H | - | 4 | - | 56 ng/ml | *L. sp.* | [79] |
| **102** | Leptosin G | $CH_2OH$ | OH | OH | $CH(CH_3)_2$ | 4 | 3 | 4.6 ng/ml | *L. sp.* | [80] |
| **103** | Leptosin $G_1$ | $CH_2OH$ | OH | OH | $CH(CH_3)_2$ | 3 | 3 | 4.3 ng/ml | *L. sp.* | [80] |
| **104** | Leptosin $G_2$ | $CH_2OH$ | OH | OH | $CH(CH_3)_2$ | 2 | 3 | 4.4 ng/ml | *L. sp.* | [80] |
| **105** | Leptosin H | $CH_2OH$ | OH | OH | $CH(CH_3)_2$ | 2 | 4 | 3.0 ng/ml | *L. sp.* | [80] |
| **106** | Leptosin I | $CH_2OH$ | H | H | - | - | - | 1.13 μg/ml | *L. sp.* | [81] |
| **107** | Leptosin J | H | $CH_2OH$ | H | - | - | - | 1.25 μg/ml | *L. sp.* | [81] |
| **108** | Leptosin K | $CH(CH_3)_2$ | OH | OH | $CH(CH_3)_2$ | 2 | 2 | 3.8 ng/ml | *L. sp.* | [82] |
| **109** | Leptosin $K_1$ | $CH(CH_3)_2$ | OH | OH | $CH(CH_3)_2$ | 2 | 3 | 2.2 ng/ml | *L. sp.* | [82] |
| **110** | Leptosin $K_2$ | $CH(CH_3)_2$ | OH | OH | $CH(CH_3)_2$ | 2 | 4 | 2.1 ng/ml | *L. sp.* | [82] |
| **111** | Leptosin M | H | OH | OH | H | 4 | - | 1.05 μg/ml | *L. sp.* | [83] |
| **112** | Leptosin $M_1$ | H | OH | OH | H | 2 | - | 1.40 μg/ml | *L. sp.* | [83] |
| **113** | Leptosin N | OH | H | H | OH | 4 | - | 0.18 μg/ml | *L. sp.* | [83] |
| **114** | Leptosin $N_1$ | OH | H | H | OH | 3 | - | 0.19 μg/ml | *L. sp.* | [83] |
| **115** | Leptosin O | $S_2CH_3$ | $CH(CH_3)_2$ | - | - | - | - | 1.1 μg/ml | *L. sp.* | [84] |
| **116** | Leptosin P | $CH(CH_3)_2$ | $S_2CH_3$ | - | - | - | - | 0.1 μg/ml | *L. sp.* | [84] |
| **117** | Leptosin Q | $SCH_3$ | $CH(CH_3)_2$ | - | - | - | - | 14.8 μg/ml | *L. sp.* | [84] |
| **118** | Leptosin R | $CH(CH_3)_2$ | $SCH_3$ | - | - | - | - | 15.2 μg/ml | *L. sp.* | [84] |
| **119** | Leptosin S | $NH_2$ | $CH(CH_3)_2$ | | - | - | - | 10.1 μg/ml | *L. sp.* | [84] |

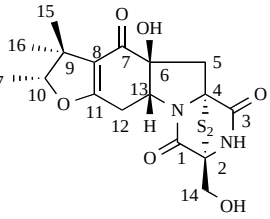
**Table 8. Structures of 120-129**



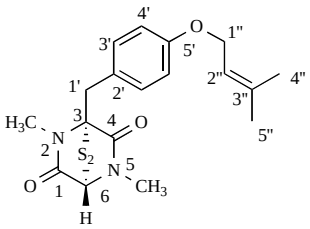
**120-122**



**123-127**



**128**



**129**

| No. | Name | $R_1$ | x | Species | Refs. |
|---|---|---|---|---|---|
| **120** | Sirodesmin A | Ac | 2 | *Sirodesmium diversum* | [85, 86] |
| **121** | Sirodesmin B | Ac | 4 | *Sirodesmium diversum* | [85, 86] |
| **122** | Sirodesmin C | Ac | 3 | *Sirodesmium diversum* | [85, 86] |
| **123** | Sirodesmin PL (Sirodesmin G) | Ac | 2 | *Sirodesmium diversum*, *Phoma lingam* | [85, 86, 87] |
| **124** | Sirodesmin H | Ac | 1 | *Sirodesmium diversum*, *Phoma lingam* | [86, 88] |
| **125** | Sirodesmin J | Ac | 3 | *Sirodesmium diversum*, *Phoma lingam* | [86, 89] |
| **126** | Sirodesmin K | Ac | 4 | *Sirodesmium diversum*, *Phoma lingam* | [86, 89] |
| **127** | Desirodesmin PL | H | 2 | *Phoma lingam* | [87] |
| **128** | Phomalirazine | - | - | *Phoma lingam* | [90] |
| **129** | Dithiosilvation | - | - | *A. silvaticus* | [91] |

and chetracin A. Compounds **96** and **98** exhibited significant antitumor activity against Sarcoma 180 ascites (%T/C 260 and 293, respectively) at doses of 0.5 mg/kg and 0.25 mg/kg, respectively [79]. Compounds **96-119** all showed potent cytotoxicity against cultured P388 cells (shown in Table **7**). It was found that dimeric ETPs (**96-98** and **102-105**) showed more potent activity than the monoeric ETPs (**99-101**) with the indole moiety, and the number of sulfur atoms in dioxopiperazine rings appered no influence on the cytotoxiciy against P388 cells. Although the configuration of sulfur bridge in **108-110** differ from that in **96-98** and **102-105**, their cytotoxic effects ($ED_{50}$) was almost identical, indicating that the configuration of sulfur bridge may not influence the cytotoxicity against P388. However, the cytotoxicities of **108-110** are much strong than those of **111-114**, indicating the disulfide or polysulfide functionalities significantly influence their cytotocixities. Further, **113** and **114** are almost 10-fold potent than those of **111** and **112** in the cytotoxic bioassay against P388, indicating that the position of hydroxyl group may influence the cytotoxicity. It may be worthy to note that **111** showed appreciable cytotoxic activities against the 39 human cancer cell lines. Moreover, evaluation of the pattern of differential cytotoxicity using the COMPARE program suggested the possibility that the mode of action for **111** might be different from that shown by any other anticancer drug developed to date [83].

## 2.8. Sirodesmins and other ETPs

Sirodesmins A-C (**120-122**), D-F (without structure reported), PL (sirodesmin G, **123**) and H-K (**124-126**) were all originally isolated from *Sirodesmium diversum* [85, 86]. Compounds **123-126** were also isolated from *Phoma lingam* [87-89] as well as deacetylsirodesmin PL (**127**) [87] and phomalirazine (**128**) [90]. The structure of **120** was determined by X-ray analysis of its diacetate derivative, and the structures of **121-123** were determined by comparison of their chemical and spectroscopic properties with those of **120**. The structure of **128**, including absolute configuration, was unambiguously elucidated by X-ray diffraction analysis. In addition, the structure of **128** has important implications on the biogenetic pathway of this broad class of sulfur bridged dioxopiperazines [90]. Dithiosilvatin (**129**), a possible key intermediate in the biosynthesis of ETP, was isolated from *A. silvaticus* [91]. Compound **129** appeared to be biosynthesized biogenetically from a compound composed from tyrosine and alanine (or serine) as in the synthesis of gliotoxin (**1**) or from syrosine and another amino acid other than glycine, and its absolute configuration was determined by comparison of its CD profile with that of emestrin (**6**). Compounds **120-123** showed exceptionally high activity against small RNA-containing virus such as the rhinoviruses and the enteroviruses [85]. For example, **120** could reduce the growth of picornaviruses in cultures of human diploid lung cells by 50% at concentration of 8 ng/ml. In contrast, the concentration of **120** required to cause 50% cytotoxicity in uninfected cultures of the same cells is 1 μg/ml. However, **120** had been shown to produce chromosome abnormalities in *in vitro* studies with human lymphocytes and in *in vivo* studies in Chinese hamster bone marrow cells. The phytotoxicity of **124**, the first reported naturally occurring monosulfur bridged dioxopiperazine, was about ten times less than that of **123** [88]. Whether the lower toxicity was due to the absence of the disulfur bridge or was

due to the lability of the monosulfide remained to be determined. Pedras *et al.* had studied the effect of acetylation on the biological activity of **123**, and found that acetylation at the 6,14-OH or 14-OH reduced the phytotoxicity considerably, whereas the phytotoxicity was not considerably affected by acetylation at the 6-OH, deducing that 14-OH may be involved in the mechanism of the toxin [89]. Rouxel *et al.* found that **123** can strongly and rapidly inhibit the incorporation [$^{14}$C]uridine in RNAs by 5.2 μM in liquid medium, and the toxicity, attributed to the reactivity of its disulphide bridge, could be reversed by the metals of the II B series (Zn, Hg and Cd). These results suggested an interaction of **123** with essential Zn from Zn-containing metalloenzymes such as RNA polymerases [92]. Boudart found Gram-positive bacteria were particularly susceptible to **123**, whereas the growth of Gram-negative bacteria was more or less affected. Meanwhile, the antibacterial activity of **123** was stronger than its monosulfide derivative and dithiomethyl-related derivative (totally inactive), demonstrating the importance of disulfide bridge of the molecule in antibacterial activity [93] (compounds **120**-**129** are shown in Table **8**).

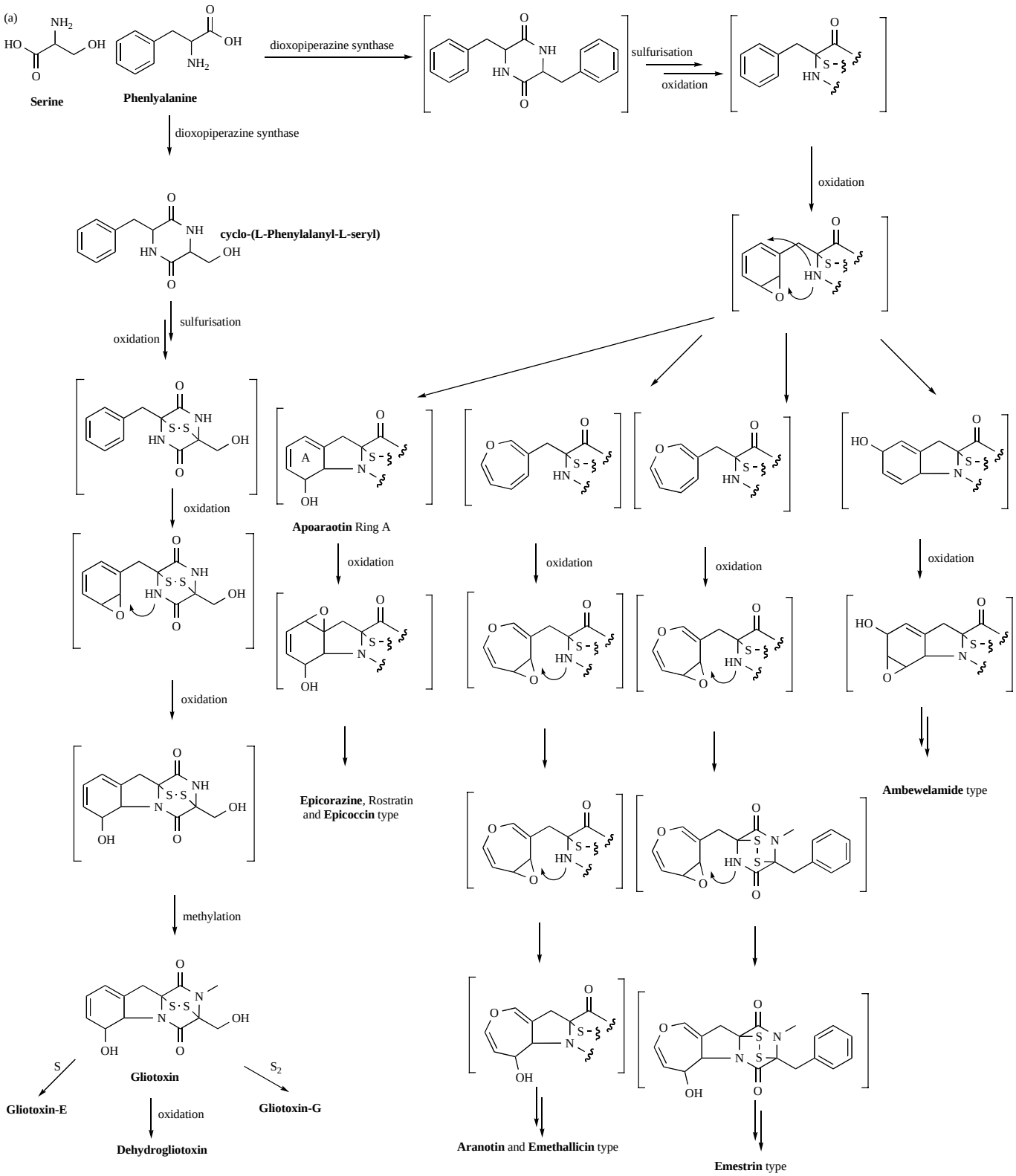
## 3. BIOSYNTHESIS

In spite of almost of 50 years of research on ETPs since the structure of gliotoxin (**1**) was first described and its biosynthesis has been being an intensive research subject, very little is known about ETPs biosynthesis [16]. Labeling and feeding experiments, the most used techniques in the biosynthetic study, were carried out leading to uncover that amino acids and cyclic dipeptides were the precursors or intermediates for gliotoxin and sirodesmin [94-99]. In the pathway to biosynthesize ETPs from *Phoma lingam*, the introduction of sulfur atoms could occur immediately following the cyclodipeptide formation, and in addition to the cyclic dipeptide phomamide two other intermediates, deacetylsirodesmin PL (**125**) and phomarilazine (**126**), have been identified [89]. Hence, the core ETP structure is formed at an early stage in the biosynthetic pathway. On the other hand, inspection of the chemical structure of **126** seems to indicate that *N*-mehtylation of the dioxopiperazine would occur after the oxidative cyclization onto the phenyl ring [90]. Introduction of the sulfur atoms in to the core ETP moiety is poorly understood. Labeling experiments suggested that the sulfur atoms in ETPs may be derived from methionine, cysteine, and sodium sulfate, however, how the sulfur atoms are introduced is unknown [16]. Genes responsible for biosynthesis of fungal secondary metabolites were found to be usually tightly clustered in the genome and co-regulated with metabolite production. Recently, the availability of identification of the putative ETP biosynthetic gene cluster is a major step forward in understanding how these molecules are produced [16, 100-102]. Herein, according to the literature surveys [2, 16, 23, 37, 44, 50, 52, 71, 90-92, 94-99], the plausible biosynthetic hypotheses of some ETPs are summarized with our meager knowledge (Fig. **1**)

## 4. ANTITUMOR ACTIVITY

The effect of ETPs on antitumor has been studied extensively and the mechanism of toxicity have been the subject of a number of reviews in 1996 and 2005 [15, 16]. Gardiner *et al.* [16] considered that the toxicity was mediated in at least two ways: (1) conjugation to proteins with susceptible thiol residues and subsequent inactivation, and (2) generation of reactive oxygen species *via* redox cycling. However, most of research on toxicity limited only to gliotoxin (**1**). The mechanism of toxicity was remains to be investigated.

Recently, some new results on antitumor activity of ETPs were reported. For instance, **1** was found to be a dual inhibitor of farnesyltransferase and geranylgeranyltansferase I with pronounced antitumor activity and favorable toxicity profile in breast cancer *in vitro* and *in vivo* [14]. Axelsson *et al.* found that glutathione promoted gliotoxin (**1**)-induced cytotoxicity in human neuroblastoma SH-SY5Y cells by reducing the ETP disulfide bridge to the dithiol form [103]. Compound **1** can induce apoptosis of cancer cells in culture and human cancer xenografts in transplant SCID mice, and this anticancer activity was further confirmed by electromicroscopic observarions [104]. Sporidesmin A (**18**) was found to inactivate human glutaredoxin (thioltransferase), a thiol-disulfide oxidoreductase, in a time- and concentration-dependent manner *via* a proposed initial reaction between the toxic sulfurs and cysteine 22 in the glutaredoxin active site [105]. This study implicates modification of sulfhydryls of target proteins in some of the cytotoxic effect of ETPs. Chaetocin (**59**) was found to be a promising compound with potent antimyeloma activity in IL-6-dependent and -independent myeloma cell lines *in vitro* and *in vivo*, and its antimyeloma activity was mediated primarily *via* imposition of oxidative stress and consequent apoptosis induction [106]. Recently, **59** and its enantiomer were found to inhibit H3K9 HMT G9a, a potential therapeutic target for treating human cancer. Both compounds were found equally effective with $IC_{50}$ values of 2.4 and 1.7 μM. In contrast, its sulfur-deficient (±)-analogues were less active ($IC_{50}$ > 50 μM) indicating that the sulfur functionality was crucial for the biological activity. Meanwhile, its enantiomer showed strong apoptosis-inducing activity through caspase-8/caspase-3 activation [107]. Staab *et al.* found that chetomin (**64**) disrupted the interaction of HIF-1, an important therapeutic target for solid tumor therapy, with the transcriptional coactivator p300. HIF-1 inhibition by **64** effectively reduced hypoxia-dependent transcription and radiosensitized hypoxic HT 1080 human fibrosarcoma cells *in vitro* [108]. The mechanistic studies revealed that **64** and other ETPs blocked the interaction between HIF-1α and p300 by a zinc ejection mechanism and the structure-activity studies revealed that the structurally unique ETP core was required and was sufficient to block the interaction of HIF-1α and p300 [109]. Gliocladicillins A (**85**) and B (**86**) were found to inhibit proliferation of HeLa, HepG2, MCF-7 tumor cells and in treated HeLa cells, to induce cell cycle arrest in the $G_2$/M phase, through activation of the p53 pathway and causing cyclin B accumulation. Compounds **85** and **86** also induced tumor cell apoptosis by activating both intrinsic and extrinsic pathways and they also exhibited significant *in vivo* anti-tumor activity in mice. These results suggest that **85** and **86** are effective antitumor agents *in vitro* and *in vivo*, and should be further evaluated for their potential in clinical


(a)
Serine
Phenlyalanine
dioxopiperazine synthase
sulfurisation
oxidation
cyclo-(L-Phenylalanyl-L-seryl)
Apoaraotin Ring A
Epicorazine, Rostratin and Epicoccin type
Ambewelamide type
Aranotin and Emethallicin type
Emestrin type
methylation
Gliotoxin
Gliotoxin-E
Gliotoxin-G
Dehydrogliotoxin

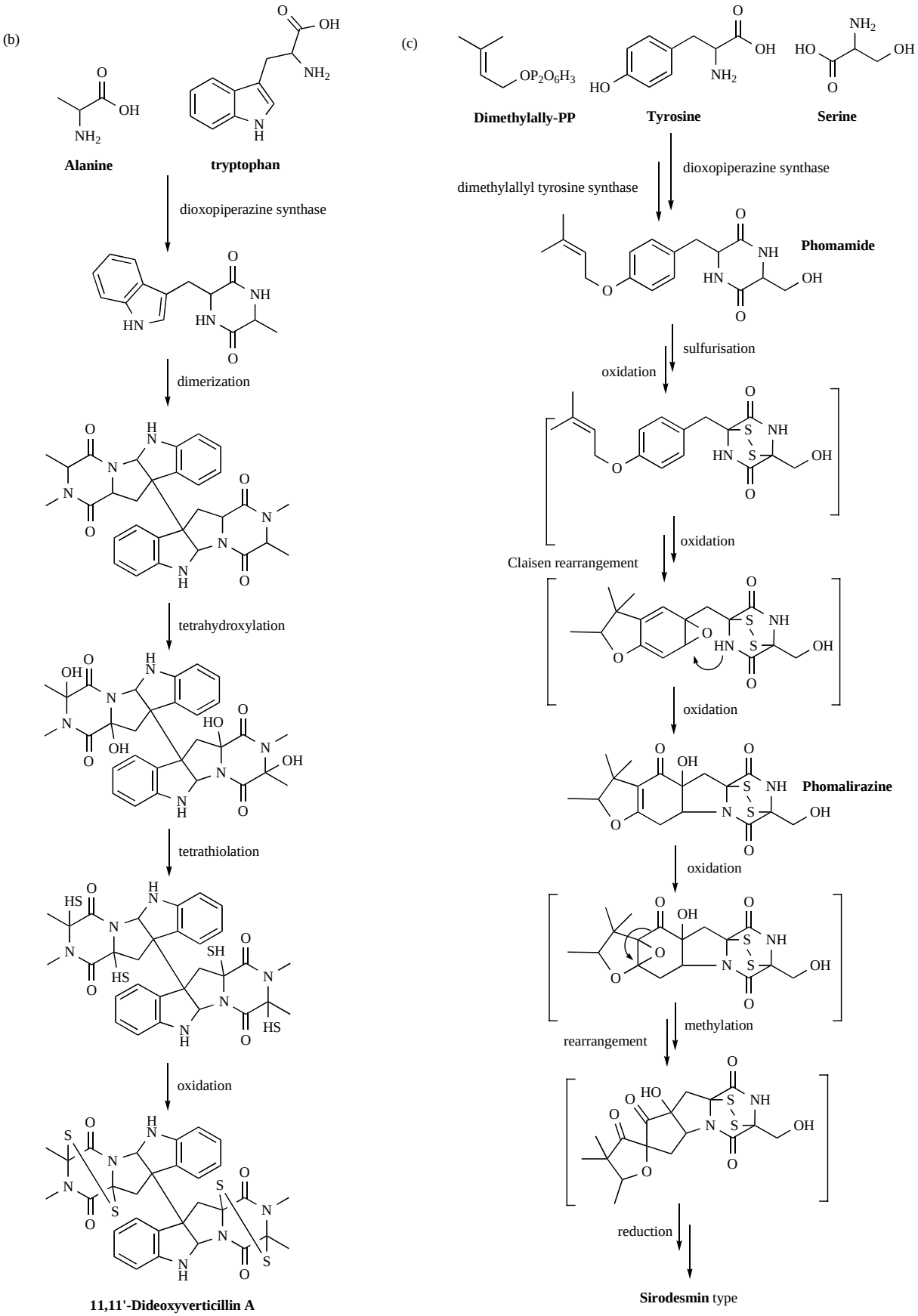



**Fig. (1).** Proposed biosynthesis hypothesis of ETPs.

usage [75]. 11,11′-dideoxyverticillin A (**75**) was found to act as a potent angiogenesis inhibitor, which inhibited angiogenesis, required for tumor growth, *via* various steps and through interfering with VEGF signal transduction [70] and to possess growth factor receptor tyrosine kinase-inhibitory effect with anti-tumor activity against four human breast tumor cell lines with an average $IC_{50}$ value of 0.2 μM [110]. Yanagihara *et al.* found leptosins C (**98**) and F (**111**) inhibited DNA topoisomerases (topo) I and/or II, targets of anticancer agents, and induced apoptosis by inactivation of Akt/protein kinase B [111].

## 5. CONCLUSIONS

To date, about 120 ETPs with broad-spectrum bioactivities have been identified from the fungi, and the members of ETPs family are still expanding. The wide range of bioactivities of ETPs are still explored extensively, and SAR studies of ETPs suggest that disulfide or polysulfide moieties play the key role for their bioactivities, which is particularly important for synthesis and design of novel anticancer drug candidates. Although it is challenging to understand the biosynthetic pathways for ETPs, the development in identification of the putative ETP biosynthetic gene cluster will make biosynthetic pathway to be understood. Meanwhile, the plausible biosynthetic hypotheses, that ETPs are derived from amino acid and cyclic dipeptides, can provide access to synthesize natural ETPs and their analogues with high bioactivity. Demand for ETPs in medicinal lead identification and in understanding biosynthesis and mechanism of activities will lead to continue the research in this field.

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## ABBREVIATIONS

CCR2 = Chemokine (C-C motif ) receptor 2

CD = Circular dichroism

DNA = Deoxyribonucleic acid

$EC_{50}$ = 50% effective concentration

$ED_{50}$ = 50% effective dose

EGF = Epidermal growth factor

HIF-1 = Hypoxia-inducible factor-1

HIV-1 = Human immunodeficiency virus type 1

hMCP-1 = Human monocyte chemoattractant protein-1

HMTs = Histone methyltransferases

$IC_{50}$ = 50% inhibitory concentration

$ID_{50}$ = 50% infective dose

IDO = Indoleamine 2,3-dioxygenase

MCP = Monocyte chemoattractant protein

MIC = Minimum inhibitory concentration

MRSA = Methicillin-resistant *Staphylococcus aureus*

MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NMR = Nuclear magnetic resonace

ORD = Optical rotatory dispersion

QRSA = Quinolone-resistant *Staphylococcus aureus*

RNA = Ribonucleic acid

SAR = Structure-activity relationship

SCID = Severe combined immunodeficiency

%T/C = Ratio of the survival time of the treated animal group (T) and that of the control group (C)

VEGF = Vascular endothelial growth factor

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