Biosynthetic Pathways and Bioactivities of Bisindole Compounds: a Short Review

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A lot of indole compounds possessing complex structures exist widely in alkaloids with enormous research significance. Specifically, bisindoles play a great role in the treatment of cancer, tumors, cardiovascular and cerebrovascular diseases. Many researchers are devoted to their biosynthesis. The structural diversity of bisindoles is attributed to multiple active sites of tryptophan. They presented bioactivities against anti-inflammatory, anti-bacterial, anti-virus and anti-cancer properties at the C-3 site of bisindoles. This paper reviews recent advances on biosynthesis pathways and bioactivities of bisindoles.

Keywords bisindoles, biosynthesis pathways, bioactivities

Introduction

A major class of secondary metabolite is alkaloid, which is nitrogen molecule derived from amino acids or purines. Among them, the particular alkaloids are derived from *L*-tryptophan. For instance, the structures of ergot alkaloids and synthetic LSD are affected by indole alkaloid *D*-levofloxacin. A recent class of *L*-tryptophan alkaloid consists of "bisindole". These molecules are produced by the fusion of two molecules containing two *L*-tryptophan indole ring in their structures. The first observation of the double indole molecule "observation" was violacein in the 1880s, which was purified and synthesized in 1958.

Staurosporine with anticancer activities, kinase inhibitors, rebeccamycin and its analogues as DNA topoisomerase I inhibitors were discovered. The terrequinone A, which has a structural relationship with antitumor, antiviral and antidiabetic compounds, has recently been isolated from fungi of the Arizona desert plant rhizosphere. The six molecules represent the only bisindole that is now available for complete biosynthesis. They have two indole rings, which are decorated in different ways such as isopentenylation, chlorination, hydroxylation and glycosylation.^[1]

The biosynthetic precursors of bisindoles are two molecules of *L*-tryptophan. Many indole molecules have been isolated from natural substances, and most of them own significant medicinal properties.^[2]

A hundreds of bisindole molecules^[3] were separated and characterized, but their biosynthetic mechanisms have been largely unexplored. This bisindole compounds have been isolated from the shawls, sponges, plant leaves, roots, bark, flowers, algae, fungi and mycotic fungi,^[3] most commonly isolated from actinomycetes,^[3] a class of bacteria known for production of secondary metabolites.^[4] In recent years, most scientists are interested in the use of bisindoles as drug molecules. They are committed to synthesizing

bisindole derivatives, testing drug properties, and separating new molecules.^[3,4]

In this review, we discussed the bioactivities and biosynthetic pathways of bisindole compounds.

Biosynthetic pathways of indoles

Biosynthetic pathways of bisindole from tryptophan

In the microbes, there are a variety of biosynthetic pathways such as polyketone, methylethylenedioxy acid, non ribosomal polypeptide, shikimic acid and heterozygous coupling of different biosynthetic pathways. The compounds produced by the heterozygous route are referred to as mixed metabolites. One of them is the mixed terpene (mixed triterpene), which presents widely in fungi, marine invertebrates and higher plants. The pentadiene pathway is conjugated with other routes by recombination.

This review describes how using the inherent reactivity of enzyme *L*-tryptophan produces divergent natural products. One characteristic of the indole ring of *L*-tryptophan is the potential reactivity of C-2 in C-2, C-5, C-6 and C-7 in the electrophilic aromatic substitution reaction. Oxidation of *L*-tryptophan to indole-3-pyruvate imide or indole-3-pyruvate increases its intrinsic reactivity. In the indole [2,3-*a*] pyrrolo [3,4-*c*] carbazole and pyridazine pathways, the conversion to indole-3-pyruvic acid imine produces an enzyme-catalyzed indole-3-pyruvate oxidized dimerized substrate imine molecules on C₆ carbon (Figure 1). The intermediates can spontaneously disintegrate to produce chromopyrrolic acid suitable for use in a series of subsequent enzymatic transformation precursors. Alternatively, in the Purple *Bacillus* route, the same intermediate may be decomposed by another route instead of forming a pyrrole ring.

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Figure 1 The pathways described how enzymes take advantage of the inherent reactivity of *L*-tryptophan to generate divergent natural products.

During the decarboxylation process, the cyclopropyl ring can be formed and disintegrated. This alternative pathway causes the migration of the indole ring [1,2], resulting in rearranging the carbon skeleton and the purple merlin precursor. Additional opportunities for dimerization and cyclization by the formation of indole-3-pyruvate from *L*-tryptophan are provided. For example, the terrequinone A biosynthetic cluster, the indole-3-pyruvate carboxylate as a thioester, binds to the carrier protein to produce an electronomer on the carbonyl carbon, which can be replaced by another indole-3-pyruvate molecules for nucleophilic attack. Thus, the rich chemistry of *L*-tryptophan provides a template for the production of various bisindole natural products.

The key site enzymes that synthesize bisindole compounds by tryptophan have been obtained, such as RebO oxidized deaminase with the site of NH₂.^[5] Rodrigues produced different expression of tryptophan gene clusters in *Escherichia coli* by metabolic engineering, such as elimination of Trp operon inhibitory genes, inhibi-

tion of trp, ser degradation pathway and synthesis of aminobenzoate (or esters) Gene TrpE feedback regulation. Biosynthesis of bisindole compounds is regulated by tryptophan gene.^[6] Further production of glycerol as a carbon source showed that the production of deoxyviolacein reached 1.6 g/L.

The components of compounds that bind at the C-3 site of indole were shown at Figure 2. The new compound **5** was derived from thiazole-substituted imidazoles in the double-indole compounds **4**, **6** and **7**. The study found that the new two compounds **6** and **7** inhibited significantly cancer cells from the human DMPM (Diffuse malignant mesothelioma) in the STO and Me-soll.^[7] Antioxidant serotonin derivatives **7–9** and dispegatrine **10** were found from *Rauwolfia*. The Dendridine A (**11**) isolated from AnOkinawan Marine Sponge *Dictyodendrilla sp.* is the only compound that binds to 4,4'-bisindole.^[8,9] Dendridine A was synthesized by chemical methods.^[10]

Figure 2 The compounds that bind at the C-3 site of indole.

The components of compounds that bind at the C-4 site of indole are shown at Figure 3.

Figure 3 The compounds that bind at the C-4 site of indole.

Biosynthetic pathways of isoindole from acetyl CoA

The formation mechanism of isoindole pyrrole ring is confirmed, and an reaction of *o*-phthalaldehyde and acetamide is taken as an example.^[11] The intermediate product was monitored every 15 min by electrospray mass spectrometry, and the presence of cyclic diols was successfully found during the reaction, but the electrospray mass spectrometer signal of this intermediate compound disappeared as the reaction progressed. However, the characteristic cleavage fragment peaks of the monocarbamic acid intermediates

were not monitored and the intermediates should only have cyclic diols in the same under the experimental conditions. By calculating the migration of monosodium carbamate [1,2]-H, [1,3] -H migration, [1,5]-H migration to the energy barrier of each reaction [1,5]-H migration of the reaction energy barrier minimum, the feasibility of such a reaction pathway should be [1,5]-H migration process.^[12] By changing the structure of the benzene ring, it can be concluded that the aroma of benzene ring can increase and decrease with the corresponding increase and decrease of energy barrier. This isoindane compounds can be synthesized through the pathway of Figure 4.

Figure 4 The reaction of *o*-phthalaldehyde and acetamide to produce isoindolinone.

A novel isoindole compound, Stachybotrin G, was found in *Stachybotrys charcarum* HDN10-123, and the biosynthetic pathway of stachybotrin was studied. To date, many similar isoindole compounds have been synthesized.^[13] The pathway of stachybotrin was shown in Figure 5.

Figure 5 Stachybotrin biosynthetic pathway.

Structures of bisindoles alkaloid from marine

At present, there are 34 bisindole alkaloids (Figure 6) derived from marine fungi, the most representative of which are leptosin compounds. Initially, the presence of leptosin compounds was found from a strain of brown algae fungi *Leptosphaeria* sp. Subsequently, 24 leptosin compounds were found from the same fungus, including leptosins A-G (12–18), G1 (19), G2 (20), H-K (21–24), K1 (25), K2 (26), M (27), M1 (28), N (29), N1 (30) and O-S (31–35). In addition to leptosins D–F (15–17), the other 21 leptosin compounds (12–14, 18–35) have a dimeric indole diketopiperazine structure, depending on the number of polysulfide bridges. The nature of the polymerization can be divided into four categories. First, the dimer diacetopiperazine structure has two polysulfide bridge structures (12–14, 18–21, 24–26); the second, dimer diacetopiperazine piperazine structure only one polysulfide bridge structure (22–23, 27–30); third, dimer diacetopiperazine piperazine structure has no poly polysulfide bridge structure (31–35); fourth, dimer diacetopiperazine piperazine structure and one indole structure polymerization

Figure 6 Structures of bisindole alkaloids 12–45.

(15–17). Leptosin compounds showed different cytotoxic activity against P388 tumor cell lines. The intensity of activity was closely related to the number of polysulfide bridge structures and their stereotaxies. In addition, leptosins A and C can inhibit the growth of rat ascites tumor cells effectively, and leptosin M has an important inhibitory effect on topoisomerase II and protein kinase PTK, CaMKIII.^[14,15] The structures of bisindole alkaloids (12–45) were shown in Figure 6.

Bioactivities of indoles

The biological activity of tryptophan derivatives was significant.^[16] A variety of bisindole compounds, such as Coscinamide, T-opsentin, and Nortaxentin, have been found in the metabolites of deep sea sponges and exhibited anti-inflammatory, antibacterial, antiviral, antibioactivity.^[17–20]

A series of bis(indole) glyoxamides have been designed and synthesized. The indole-3-glyoxylic acid chloride formed in the reaction of indole and oxalyl chloride, which was easily obtained by treatment with tryptamine to give 82%–93% bis(indolyl) glyoxylamide. All synthetic bis(indole) glyoxyl amides were fully characterized and tested for their antimicrobial activity against Gram-positive and Gram-negative bacterial strains. Some compounds exhibited potent antimicrobial activity against Gram-negative strains.

In addition, the cytotoxicity of bis(indole) glyoxylamide was evaluated against a group of human cancer cell lines. PC-3 cells exposed to compounds will result in increased levels of cleaved PARP1, which suggests that bis(indole) glyoxylamide induces PC-3 cell apoptosis. Most importantly, some compounds were completely absent in mammalian cells, indicating that they targeted the bacterial specific target and therefore did not show any toxicity in the host cell.^[21]

Bioactivities of indoles from marine

Marine alkaloids, especially indole alkaloids, not only had a very unique and complex chemical structure, but also showed strong anti-tumor, anti-virus, anti-bacterial and anti-inflammatory and other physiological activities (Table 1).

Asperazine (36) is a cytotoxic, asymmetric structure of dimeric indolizine piperazine alkaloids, derived from a Florida sponge *Hyrtios proteus* epiphytic fungi *Aspergillus niger*.^[22] The biggest feature of its structure is that the two diketopiperazine monomers are linked to a benzene ring carbon in a tryptophan structure, unlike the leptosin compound non-aromatic carbon dimerization.

Two dimeric indole diketopiperazine compounds 11, 11-dideoxyverticillin A (37) and 11-deoxyverticillin A (38) with a disulfide bridge structure were isolated from *Penicillium sp.* (CNC-350) derived from Caribbean green algae. These two compounds showed significant cytotoxic activity in human colon cancer cell line HCT-116 *in vitro*, with an IC₅₀ (half maximal inhibitory concentration) value of 30 µg·mL⁻¹.^[23]

The structure of the compound 39 is asymmetric. After the acid hydrolysis, the chiral HPLC was performed to determine the two diketopiperazine monomer containing D-amino and D-leucine.^[24]

Glioclads A–C (40–42) and glioperazine with cytotoxic activities are derived from the strain of fungi *Gliocladium sp.*, and they are structurally polymerized from a single indole structure with one diketopiperazine monomer.

The gliocladin C showed a relatively strong cytotoxic activity against P388 cell lines with an ED₅₀ (50% effective dose) of 2.4 µg·mL⁻¹.^[25] The diol indole dipeptide piperazine alkaloid plectosphaeroic A–C (43–45) were isolated from one marine fungus *Plectosphaerella cucumerina* showing activities with IDO (indoleamine 2, 3-dioxygenase) inhibition by activity tracing.^[26]

Table 1 Bisindole alkaloids from marine-derived

No.	Activity	Name of strain	Source of strain
12-35	Cytotoxic activity	<i>Leptosphaeria sp</i>	<i>Sargassum tortile</i>
36	Cytotoxic activity	<i>Aspergillus niger</i>	<i>Hyrtios proteus</i>
37-38	Cytotoxic activity	<i>Penicillium sp.</i>	<i>Avrainvillea longicaulis</i>
39	Nosignificant inhibition of viral ribosome entry into the site of the translation activity	<i>Aspergillus niger</i>	Unknown source
40-42	Cytotoxic activity	<i>Gliocladium sp.</i>	<i>Aplysia kurodai</i>
43-45	Indoleamine-2,3-double-plus-oxidase inhibitor	<i>Plectosphaerella cucumerina</i>	Sediment

Bioactivities of indoles not from marine

(3'R)-hydroxytaptanate C (46) was isolated from the cytotoxic alkaloid fraction of the methanol extract of *Tabernaemontana elegans* by fractionation, which is a fobasic acid-iboga bisindole alkaloids. Its structure was identified by spectroscopic methods, mainly 1D and 2D NMR experiments. Compared with the most commonly used anticancer agents in colorectal cancer treatment 5-fluorouracil (5-FU), (3'R)-hydroxytaberane-legantine C (46) showed strong apoptosis-inducing activity. In the MTS assay, compound 46 showed IC₅₀ values that were similar or deficient to 5-FU in the three cell lines tested. Lactate dehydrogenase assay showed an increase in LDH (lactate dehydrogenase) release for compound 46, and the guava ViaCount assay showed a significant increase in the incidence of compound 46 to 5-FU cell apoptosis. Moreover, the induction of apoptosis was corroborated by evaluation of nuclear morphology by Hoechst staining and caspase-3/7 activity assays of compound 46 treated. As expected, the treatment of compound 46 resulted in poly (ADP-ribose) polymerase cleavage in immunoblot analysis. The levels of anti-apoptotic protein Bcl-2 and XIAP steady state were reduced in all three cancer cell lines tested. It is possible that compound 46 exhibits significant apoptosis induction in HCT116, SW620 and HepG2 cells. The results show that compound 46 is a promising lead structure that induces apoptosis.^[27]

Melodinine V (47) with the vinblastine-ylidene skeleton was isolated from *Melodinus henryi*. The structure was clarified by a broad spectrum method and further confirmed by single crystal X-ray diffraction analysis. Melodinine V showed selective cytotoxicity against human colon cancer cell line HT-29 and inhibited cell proliferation in a concentration-dependent manner. Proportion of cell cycle arrest and apoptosis in G1 phase were induced by increasing histone-associated DNA cleavage in treated HT-29 cells.^[28]

Bisindole-PBD (48) was evaluated for the anti-angiogenic activity in MCF-7 and MDA-MB-231 cell lines. The main method was to use the formation of *in vitro* endothelial cells (HUVEC) tube, indicating the role of 48 during the process of breast cancer cells wound healing *in vitro*. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) was also performed to examine the expression of VEGF in breast cancer cells and HUVEC cells. A horioallantoic membrane (CAM) assay was performed to observe the effect of bisindole-PBD on angiogenesis in chicken embryos. Bisindole-PBD can regulate breast cancer cell proliferation by inhibiting angiogenesis; moreover, it inhibited

VEGF expression at both transcriptional and post-transcriptional levels. In addition, the level of STAT3 was significantly downregulated after drug treatment, which displayed that STAT3 was associated with VEGF transcription. The expression of MMP-2 and MMP-9 was also regulated by compound **48**. The *in vivo* study of the chick chorioallantoic membrane (CAM) showed an anti-angiogenic effect, which was consistent with *in vitro* data. In general, compound **48** acts as an effective small molecule with anti-angiogenic activity.^[29]

Four new eburnamine-aspidospermine type bisindole alkaloids mekongenines C–F (**49–52**) and 27 known indole alkaloids were isolated from the foliage and leaves of *Bousigonia mekongensis*. Their structures with absolute configuration are elucidated by spectral method and ECD (electrochemical detection) analysis. HL-60, SMMC-7721, A-549, MCF-7 and SW480 were evaluated for their cytotoxicity *in vitro*. Alkaloid **49–52** showed inhibitory effect, whose IC₅₀ values were equivalent with that of cisplatin.^[30]

A series of different benzofuran dioindole hybrid systems were synthesized in triton WR-1339-induced hyperlipidemia rats for antioxidants *in vitro* and antibody lipid activities *in vivo*. In this series, compounds **53–56** showed a significant reduction in plasma levels of total cholesterol (TC), phospholipid (PL) and triglyceride (TG), followed by an increase in heparin lipolytic activity (PHLA). In addition, the active hybrids had moderate antioxidant properties and increased plasma lecithin cholesterol acyltransferase (LCAT) activity, which plays a key role in lipoprotein metabolism and contributes to elevated levels of HDL-C in serum.^[31]

Two new alkaloids, Hyrtimomines D (**57**) and E (**58**), were isolated from Okinawa Ocean Sponge *Hyrtios* sp. The structures of **57** and **58** were elucidated by spectral analysis. The thiamethoxamate D and E are structurally unique bisindole alkaloids having a keratin-6-one skeleton with hydroxyindole and imidazole units. Their activities of inhibiting *Cryptococcus neoformans* were IC₅₀ of 4 and 8 µg/mL, respectively. Hyrtimomine D showed inhibitory activity against *Staphylococcus aureus* (MIC (minimal inhibitory concentration)=4 µg/mL) and *Trichophyton mentagrophytes* (MIC=16 µg/mL).^[32]

Conclusion

Bisindole compounds have a indole core and diverse substituent groups, but they are mainly synthesized through the tryptophan pathway and are regulated by the tryptophan synthesis gene. The tryptophan is purified by oxidation, polymerization, and other reactions for biosynthesis of a series of active double indole compounds. Another biosynthetic pathway initiated by acetyl-CoA is long, and it did not form a bisindole compound but form a monoindole compound with a long hydrocarbon chain eventually. Some biosynthetic pathways of indole compounds have been confirmed by previous researches, such as stachybotrin and Violacein. Numerous indole compounds have been developed, but their biosynthetic pathways have not been explored such as isoindolone. Bisindoles would be a hot issue in the field of medicinal chemistry in the future.

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References

- [1] Katherine, S. R.; Catherine, L. D. *J. Chem. Biol.* **2011**, *16*, 351.
- [2] Katherine, S. R.; Catherine, L. D. *Nat. Prod. Chem. Biol.* **2009**, *16*, 351.
- [3] Higuchi, K.; Kawasaki, T. *Nat. Prod. Rep.* **2007**, *24*, 843.
- [4] Prudhomme, M. *Curr. Med. Chem.* **2004**, *4*, 509.
- [5] Sanche, C.; Brana, A.; Mendez, C.; *Chembiochem.* **2006**, *07*, 1231.
- [6] Alkhalaf, L.; Ryan, K.; *Chem. Biol.* **2015**, *22*, 317.
- [7] Shimizu, S.; Yamamoto, Y.; Inagaki, J.; *Gan.* **1982**, *73*, 642.
- [8] Parrion, B.; Carbone, A.; Vita, G.; Ciancimino, C.; Attanzio, A.; *J. Chembiochem.* **2015**, *13*, 460.
- [9] Lin, M. Yang, B. Qi; Yu, D. Q. *Acta. Pharm. Sinica* **1986**, *21*, 114
- [10] Tsuda, M.; Takahashi, Y.; Fromint, J.; Mikami, Y.; Kobayashi, J.; *J Nat Prod.* **2005**, *68*, 1277.
- [11] Zhang, Y.; Gou, L.; Yang, G.; Guo, F.; Di, Y.; *Fitoterapia.* **2015**, *100*, 150.
- [12] Wan, J.; Wu, B.; Pan, Y.; *Cheminform.* **2007**, *38*, 9338.
- [13] Song, K.; Hu, J.; Lin, W.; Ji, Y.; *Biotechnology Bulletin.* **2014**, *04*, 36.
- [14] Takahashi, C.; Numata, A.; Ito, Y.; *J Chem Soc Perkin Trans I.* **1994**, *13*, 1859.
- [15] Yamada, T.; Iwamoto, C.; Yamagaki, N.; Yamanouchi, T.; Minoura, K.; Yamori, T.; Uehara, Y.; Andoh, T.; Umemura, K.; Numata, A. *Tetrahedron* **2002**, *58*, 479.
- [16] Rodrigues, A. L.; Trachtman, N.; Becker, J.; Lohanatha, A. F.; Blothenberg, J.; Bolten, C. J.; Korneli, C.; Lima, A. O. D.; Porto, L. M.; Sprenger, G. A.; Wittmann, C. *Metab. Eng.* **2013**, *20*, 29.
- [17] Liu, Y. F.; Chen, M. H.; Wang, X. L.; Guo, Q. L.; Zhu, C. G.; Lin, S.; Xu, C. B.; Jiang, Y. P.; Li, Y. H.; Jiang, J. D.; Li, Y.; Shi, J. G. *Chin. Chem. Lett.* **2015**, *26*, 931.
- [18] Bokesch, H. R.; Pannell, Lewis. K.; McKee, T. C.; Boyd, M. R. *Tetrahedron. Lett.* **2000**, *41*, 6305.
- [19] Shimizu, S.; Yamamoto, Y.; Inagaki, J.; Koshimura, S. *Gan* **1982**, *73*, 642.
- [20] Bartik, K.; Braekman, J. C.; Daloze, D. *Can. J. Chem.* **1987**, *65*, 2118.
- [21] Mukund, P.; Tantak, V. G.; Kumar, Nikhil. V.; Arun, R. P. S.; Prabhat, N. J.; Kavita, S. D. K. *ACS Med. Chem Lett.* **2016**, *26*, 3167.
- [22] Angela, P.; Sofia, E.; Gomes, P. M.; Borralho, M. B.; Silva, M.; Cecilia M.P. Rodrigues, M. U. *Ethnopharmacol.* **2016**, *194*, 236.
- [23] Liu, Y. P.; Grace, G. L. Y.; Julia, K. M. L.; Feng, T.; Zhao, Y. L.; Li, Y.; Clara, B. S. L.; *Bioorg. Med. Chem. Lett.* **2016**, *26*, 4895.
- [24] Pranjali, S. M.; Janaki, R.; Dhananjaya, P.; Utpal, B.; Manika, P. B. *Life Sci.* **2016**, *151*, 264.
- [25] Fu, Y. H.; Li, S. L.; Li, S. F.; He, H. P.; Di, Y. T.; Zhang, Y.; Hao, X. J. *Fitoterapia* **2014**, *98*, 45.
- [26] Koneni, V. S.; Ram, K. M.; Ravi, S.; Bhaskara, R. K.; Gitika, B.; *Eur J Med Chem.* **2013**, *68*, 38.
- [27] Naonobu, T.; Rei, M.; Yohei, T.; Takaaki, K.; Azusa, T. N.; Tohru, G.; Jane, F.; Jun, K. *Tetrahedron Lett.* **2013**, *54*, 4038.
- [28] Wang, F.; Fang, Y.; Zhu, T. *Tetrahedron* **2008**, *64*, 7986.
- [29] Son, B. W.; Jensen, P. R.; Kauffman, C. A.; Fenical, W. *Nat. Prod. Lett.* **1999**, *13*, 213.
- [30] Ovenden, S. P.; Sberna, G.; Talt, R. M.; Wildman, H. G.; Patel, R.; Li, B.; Steffy, K.; Nguyen, N.; Meurer-Grimes, B. X. *J. Nat Prod.* **2004**, *67*, 2093.
- [31] Usami, Y.; Yamaguchi, J.; Numata, A. *Heterocycles* **2004**, *63*, 1123.
- [32] Carr, G.; Tay, W.; Bottrill, H.; Andersen, S. K.; Mauk, A. G.; Andersen, R. J. *Org. Lett.* **2009**, *11*, 2996.