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REVIEW ARTICLE

A Brief Review on Structure-Bioactivity Relationship Study of Ophiobolins and Derivatives

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Abstract

Ophiobolins show a broad-spectrum inhibitory activity against nematodes, fungi, bacteria, and cytotoxic activity. Now many ophiobolin and their derivatives have been identified, nevertheless, there are few studies on structure-activity relationships for this class of compound. This review aims at summarizing published researches of ophiobolins and their derivatives, including the source, structure, bioactivity, the mode of action and the possible factors that cause the different bioactivity. In view of the fact that the activity of ophiobolins were generally higher than 6-epi-ophiobolins, ophiobolin O, K and 6-epi-ophiobolin O, K were studied as an example. The geometries, bond lengths, bond orders of the four compounds have been investigated at B3LYP/6-31+G(dip) level by density functional theory (DFT). The DFT analysis show that the ophiobolins steric of function group related to C (7) were much lower than 6-epi-ophiobolins, it suggested that C-H was the key bioactive group. The low steric reactive function group can be made to maximize contact with the target and thereby enhance the biological activity. It proposed a new pathway for the design and synthesis of the active molecule.

1. Diversity of ophiobolins

Ophiobolins are naturally occurring sester-terpenes characterized by an unusual tri-cyclic 5-8-5 ring system, some of them have an extra ring incorporated, forming different types of tetra-cyclic structures^[1-3]. Ophiobolin A was the first member isolated from *Ophiobolus Miyabeanus*^[4], and characterized independently by Canonica et al.^[5] and Nozoe et al.^[6]. Currently, ophiobolin A to ophiobolin W and many analogues were isolated and identified, these include ophiobolin B from *Bipolaris oryzae*^[7], ophiobolin C from *Bipolaris zizanie*^[6], ophiobolin D from *Cephalosporium caerulens*^[8-9], ophiobolin E from *Drechslera gigantean*^[10], ophiobolin F from *Bipolaris maydis*^[11], ophiobolin G and H from *Aspergillus ustus*^[12], ophiobolin I and J from *Drechslera oryzae*^[13], ophiobolin K from *Aspergillus ustus*^[14], ophiobolin L from *B. maydis*^[2], ophiobolin M from *Cochliobolus heterostrophus*^[15], ophiobolin O from *Aspergillus ustus*^[16], ophiobolin P-T from *Ulocladium sp*^[17], ophiobolin U-W from *Aspergillus ustus*^[18]. Among most of them were isolated from land sources, while some of them were produced by marine microorganisms^[19]. In addition, it has been proved the production of ophiobolins and derivatives by the fungi seems to change with the culturing conditions^[2], ophiobolin can also be degenerated into derivatives in culture media^[20].

2. Biological actions of ophiobolins

Ophiobolins and their derivatives have good bioactivities, such as Anticancer, Nematocidal Activity, Apoptosis-inducing, Herbicidal, Antifungal and Antimicrobial activities^[21-23], some of them have been

applied in clinic. Except for bioactivity research, relevant work has been carried out to find out the active mechanism, including the synthesis of ophiobolin^[24], the construction of core unit like 5-8 bicyclic systems, 5-8-5, 5-8-6-5 rings and spirocyclic CD-ring moiety^[25-29].

At present, The research of bioactivities of ophiobolins and derivatives mainly covers the antitumor activities (cytotoxicity), phytotoxicity, antifungal and antimicrobial activities. The mechanism of action on cell death can be triggered by a range of intracellular stresses such as DNA damage, oxidative stress, cytosolic Ca²⁺ overload and the accumulation of misfolded proteins^[30]. While it is still a challenge to understand exactly the mechanism of action of ophiobolin, such as how the composite molecule inhabits or kills cancer cells. Among them, ophiobolin A was widely studied for its significant activities.

2.1. Antitumor activities (Cytotoxicity).

Ophiobolin A showed cytotoxicity in the L1210 cell line^[31], inhibited solid and haematological cancer cell proliferation, induced apoptosis and inhibited cell cycle progression in MDA-MB-231 cancer cells^[32]. Ophiobolin A can be incorporated into novel chemoembolization particles for the reduction in cell viability of rhabdomyosarcoma cancer (RD) cells^[33], also can inhibit growth even induce cell death of several mammalian cancer cell lines irrespective of their multidrug resistance phenotypes and their resistance levels to pro-apoptotic stimuli^[20]. Ophiobolin A strongly reduced cell viability of melanoma A375 and CHL-1 cell lines, by affecting mitochondrial functionality^[34]. Ophiobolin A inhibited the proliferation and migration of GBM cells,

by inhibiting big conductance Ca^{2+} -activated K^+ channel activity, also induced paraptosis-like cell death in GBM cells, by the swelling and fusion of mitochondria and/or the endoplasmic reticulum of GBM cells^[35]. Ophiobolin A reacts with the ethanolamine head group of phosphatidylethanolamine (PE) in human cells and form pyrrole-containing covalent cytotoxic adducts, these adducts lead to lipid bilayer destabilization. At the same time, the genetic inactivation of de novo synthesis of PE can reduce cellular PE levels, then mitigate cytotoxicity of ophiobolin A^[36]. Ophiobolin A lead to an arrest of the cell cycle and alter the intracellular partitioning of glutathione between the nuclei and cytoplasm of TBY-2 cell^[37]. Ophiobolin A shows the acute effect on HO-mediated inflammatory processes, caused a significant elevation in the concentrations of IL-6 and TNF- α , increased MPO activity and decreased HO enzyme activity in the plasma^[38]. Ophiobolin A can cause protein misfolding and the accumulation of misfolded proteins, leading to paraptosis-like cell death in glioblastoma cells^[39]. Ophiobolin A displays different mechanisms of cell death in mammalian cells depending upon the cancer cell origin, and the mitochondria appear to be a central player in ophiobolin A-induced cancer cell death^[40]. Ophiobolin A showed activity against cancer cell-lines A-549, HT-29, and Mel-20 at IC_{50} (50 ng/mL) and cell-line P-388 at IC_{50} (25 ng/mL), also showed potent marginal activity against *P. falciparum* at IC_{50} (ng/mL) <528.8 (D6 Clone, SI >2.1), 580 (W2 Clone, SI 1.9) in an antimalarial assay^[41]. Ophiobolin A exhibited strong motility inhibition and viability reduction on boar spermatozoa and significantly damaged the sperm

mitochondria at sublethal concentration by the dissipation of transmembrane potential in the mitochondrial inner membrane, also highly toxic to cell proliferation in MNA, FFL and PK-15 somatic cell lines under the level causing motility inhibition on boar spermatozoa^[42].

3-anhydro-6-hydroxy-ophiobolin A induced autophagy and promoted the degradation of α -synuclein in PC12 cells, possessed strong autophagy-inducing activity to GFP-LC3 stable HeLa cells, also induce autophagy both in U2OS and SH-SY5Y cells^[43]. Ophiobolin O reduced the viability of human breast cancer MCF-7 cells in a time-and dose-dependent manner and efficiently induced apoptosis in MCF-7 cells. In vivo, ophiobolin O suppressed tumor growth and showed little toxicity in mouse xenograft models^[16, 44]. Ophiobolin H induced hyperacusia in day-old chicks at rates up to 375 mg kg^{-1} , but there was no mortality^[12]. Ophiobolin P-T, 6-epi-21, 21-O-dihydrophiobolin G, 6-epi-ophiobolin G and 6-epi-ophiobolin K showed strong cytotoxicity against two cell lines including KB and HepG2 cell line in comparison with the positive control etoposide. Ophiobolin T and 6-epi-ophiobolin G exhibited stronger cytotoxic activities against HepG2 than etoposide with IC_{50} values of 0.24 and 0.37 μM , respectively^[17]. Ophiobolins B and J showed phytotoxicity on various four weed species at the concentration of 0.5 mg mL^{-1} , while ophiobolins E and 8-epi-ophiobolins J appeared to be inactive^[10]. Ophiobolin U displayed >75% lethality at 100 $\mu\text{g mL}^{-1}$ and LC_{50} values of 48.1 $\mu\text{g mL}^{-1}$ in the brine shrimp toxicity assay^[18]. Ophiobolin K showed cytotoxicity against tumor cell lines, including adriamycin-resistant mouse leukemia cells (P388),

with IC_{50} of 0.27–0.65 μM . Especially, it showed seven times stronger cytotoxic activities against P388/ADR (adriamycin resistant cells) tumor cells than adriamycin [22].

2.2. Phytotoxicity. Ophiobolin A shows good stimulatory effect to *O. aegyptiaca*, *O. cumana*, and *O. minor*, these broomrapes were stimulated up to 50% in the concentration range of 10^{-4} – 10^{-7} M of A [45]. Ophiobolin A prevented cell proliferation even induced cell death in *Nicotiana tabacum* L. cv. Bright Yellow 2 (TBY-2) cells depending on the concentrations. *In vitro* growth-inhibitory assays on four cancer cell lines (A549, SKMEL28, Hs683 and B16F10), the mean IC_{50} growth inhibitory concentrations for 3-anhydro-6-*epi*-ophiobolin A were approximately 60 times higher than those for ophiobolin A [20]. Ophiobolin A can mutate Lys-75, Lys-77, and Lys-148 residues in calmodulin molecule by site-directed mutagenesis, while Lys residues in positions 86 and/or 143 which are highly conserved in plant calmodulins did not react with ophiobolin A [46]. Furthermore, kinetic analysis provide that lysine 75 in calmodulin is the primary inhibitory site for ophiobolin A [47].

Ophiobolin A inhibit spinach calmodulin, and the calmodulin may be one of the target proteins of the phytotoxic action of ophiobolin A [48]. Ophiobolin A inhibit the phosphorylation of S6 (IC_{50} = 1.9 ± 0.2 μM), ERK (IC_{50} = 0.28 ± 0.02 μM) and RB (IC_{50} = 1.42 ± 0.1 μM), the effector proteins of PI3K/mTOR, Ras/Raf/ERK and CDK/RB pathways, respectively [31]. Ophiobolin A could induce hyphal malformation in *Phytophthora capsici*, the effect may be due to the inhibition of β -1, 3 glucan synthetase

similarly [49].

Ophiobolin B inhibited proton extrusion from maize coleoptiles by stimulating electrolites, glucose and aminoacid leakage. Moreover, ophiobolin B counteracts the biological activity of fusaric acid (FA), inhibits FA-proton extrusion, potassium uptake and cell enlargement [50]. Ophiobolin G and ophiobolin H inhibited etiolated wheat coleoptile growth [12]. 6-*epi*-ophiobolin I, ophiobolin J, 8-deoxyophiobolin J, and ophiobolin I can cause yellowish to reddish lesions on rice cultivars in a nonselective manner at 10^{-4} M, also effectively reduce, by 50%, dark CO_2 fixation in rice leaf pieces in the range of 10^{-4} – 10^{-5} M [13].

2.3. Antifungal and antimicrobial activities.

Ophiobolins A and B showed antifungal activity on zygomycete with MIC values of 3.175–50 $\mu g\ mL^{-1}$ and 25–50 $\mu g\ mL^{-1}$, respectively. Ophiobolin A inhibited sporangiospore germination of *mucor circinelloides* and caused morphological changes [51]. The minimum inhibitory concentration of ophiobolin A against *Candida albicans*, *Aspergillus flavus*, *Trichopbyton mentagropbytes*, *Torulopsis cremoris*, *torulopsis petropbilum* is 12.5, 25, 12.5, 0.20 and 1.56 $\mu M\ mL^{-1}$, respectively [2,52]. 3-Anhydro-6-hydroxy-ophiobolin A displays high efficacy against influenza A virus infection in vitro and in vivo by inhibiting influenza virus replication and enhancing the host immunity [53].

Ophiobolin P and T showed moderate antibacterial activity against *B. subtilis* and methicillin-resistant *S. aureus*. Ophiobolin T and 6-*epi*-ophiobolin G exhibited the cytotoxic activity against HepG2 with IC_{50} of 0.24 and 0.37 μM , respectively [17]. Ophi-

obolin G and ophiobolin H inhibited growth of *Bacillus subtilis* cultures, but ophiobolin H was a more potent inhibitor at rates above 250 µg/disk than G^[12]. Ophiobolin U and (5α,6α)-ophiobolin H exhibited inhibitory activities against *Escherichia coli* with inhibitory diameters of 15 and 10 mm, respectively, ophiobolin U also showed activity against *Staphylococcus aureus* with inhibitory diameters of 10 mm at 30 µg disk⁻¹^[18].

Ophiobolin K showed high inhibitory activities against *Aspergillus repens* and *Aspergillus glaucus* with MIC value of 0.78 µg mL⁻¹, Alao exhibited potent activity against gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*) with MIC values of 0.78, 1.6 and 3.1 µg mL⁻¹^[54]. In comparison, tetracycline inhibits the growth of both gram-positive and gram-negative bacteria with MIC of 8 µg mL⁻¹^[55]. At 75 µM, ophiobolin K exhibited 81.9, 84.5, and 79.9% growth inhibition of *C. acutatum*, *C. gloeosporioides* and *Fusarium oxysporum* after 48h, respectively, and caused 86.9% growth inhibition of *Phomopsis viticola* after 120h. At 150 µM, 6-epiophiobolin K showed 96% growth inhibition of *P. obscurans* after 120 h^[14].

2.4. Activities of ophiobolins and 6-epi ophiobolins. While summarizing the bio-activities of ophiobolins, a conclusion can be obtained. The activities of ophiobolins with β-stereochemistry at C-6 position were generally higher than α-stereochemistry, some of them even showed big difference.

In the potency of inhibiting maize calmodulin *in vitro* and the effects on excised roots assay, ophiobolin A is better than 6-epi-ophiobolin A^[56]. In the leaf puncture assay to several monocotyledons and dicotyledons, ophiobolin A is more active in comparison with 6-epi-ophiobolin A^[57]. Ophiobolin K and 6-epi-ophiobolin K showed trypanocidal activities with IC₅₀ values of 13.0 and 9.62 µM against *T. cruzi*, this is approximately four and two times higher in comparison with the drug control benznidazole (3.84 µM)^[14, 58].

In the nematocidal activity in a *C. elegans* motility assay, ophiobolin C shows the most active with an LD₅₀ value of 5 µM, ophiobolin M and ophiobolin K were similarly active with LD₅₀ values of 13 and 26 µM. While epi-ophiobolin C, M, K with LD₅₀ values of 130, 130 and more than 260 µM, respectively (Table 1)^[2,15,59].

Table 1. Inhibition of [³H] ivermectin binding and biological potencies on *C. elegans* motility in vivo for ophiobolins.

Number	Compound	Binding(<i>K_i</i> µM)	Motility(LD ₅₀ µM)
1	Ophiobolin M	31	13
2	Epi-ophiobolin M	156	130
3	Ophiobolin C	15	5
4	Epi-ophiobolin C	180	130
5	Ophiobolin K	130	26
6	Epi-ophiobolin K	260	>260
7	Dihydroophiobolin C	45	25

In the cytotoxic activity assay towards CLL cells, ophiobolin A, B, C, and K showed the strongest effects with LC₅₀ values between 1 and 8 nM, while some derivatives were

inactive, ophiobolin K and 6-epiophiobolin K showed effects with LC₅₀ of 4 nM and Inactive (**Table 2**)^[3].

Table 2. Apoptosis inducing activity of the ophiobolins towards CLL cells.

Compound	LC ₅₀ (nM)
Ophiobolin A	1 nM
3-anhydro-ophiobolin A	Inactive
3-anhydro-6-epi-ophiobolin A	Inactive
Ophiobolin B	2 nM
Ophiobolin C	8 nM
6-epiophiobolin G	Inactive
Ophiobolin H	Inactive
Ophiobolin K	4 nM
6-epiophiobolin K	Inactive
6-epiophiobolin H	Inactive

In the antifungal and antimicrobial activities assay, ophiobolin L showed better activity than 6-epi-ophiobolin L against most of fungi except for the *Staphylococcus aureus* ^[46]. Ophiobolin K displayed an IC₅₀ of 0.51 µM and 6-epi-ophiobolin an IC₅₀ of 2.97 µM against the both tumoral cells TK-10 and MCF-7, respectively ^[14].

The anti-biofilm activity of ophiobolin K and 6-epi-ophiobolin K were 4.1 and 65µM, respectively, also ophiobolin K showed antibiotic activity to mycobacterium smegmatis and mycobacterium bovis of 8.2 and 64 µM, respectively, but 6-epi-ophiobolin K

was inactive ^[60-61].

3-anhydro-6-hydroxy-ophiobolin A(1) exhibited potent antiproliferative activity against cell lines HepG2 and K562 with IC₅₀ of 6.49µM and 4.06µM, respectively, while 3-anhydro-6-epi-ophiobolin A(3) showed weak activity with IC₅₀ of 47.1µM, 35.6µM, respectively. Also A(1) presented moderate antifungal activity against *Candida albicans* and strong antimicrobial activity against *Bacille Calmette–Guerin*, *Bacillus subtilis*, *Staphylococcus aureus*, while A(3) showed weak activity in these assays with MIC value larger than 100 µg mL⁻¹ (**Table 3**) ^[62].

Table 3. Cytotoxicity and antimicrobial activity of compounds 1, 2, 3.

Compounds	MIC(µg/mL)						IC ₅₀ (µM)	
	BCG	BS	PA	SA	MRSA	CA	HepG 2	K562
1	12.5	12.5	> 100	12.5	12.5	50	6.49±0.34	4.06±0.50
2	> 100	> 100	> 100	> 100	> 100	> 100	55.7 ± 3.8	39.5± 5.5
3	> 100	> 100	> 100	> 100	> 100	> 100	47.1± 4.8	35.6± 4.4
Positive control	isoniazid	vancomycin	ciprofloxacin	vancomycin	vancomycin	ketoconazole	Etoposide	
	0.05	0.50	1.00	1.00	1.00	0.02	6.41± 0.50	

3. Views of structure-bioactivity relationship

3.1. Functional group

Some studies showed, functional group causes the different activity of ophiobolins

and derivatives, such the hemiacetal structure in ophiobolin H is more inhibitory than the ketoaldehyde structure in ophiobolin G in inhibiting the growth of wheat coleoptiles (**Fig. 1**)^[2,12].

Fig. 1. Structure of Ophiobolin H and G.

In the antibacterial activity assay of ophiobolins and derivatives, ophiobolin T showed stronger in both cytotoxic and antibacterial activities, while others compounds showed bioactivities only in part

cell lines, which suggests that the furan ring formed in the side-chain at C-15 significantly influences the bioactivities (**Fig. 2**)^[17].

Fig. 2. Structures of opiobolins and derivatives, opiobolin T with furan ring.

According to bioactivity assays (**Table 3**), 3-anhydro-6-hydroxy-opsiobolin A(1) showed better activity than 3-anhydro-opsiobolin A(2) and 3-anhydro-6-epi-opsiobolin A(3), it was considered the in-

roduction of a hydroxyl group at C-6 in 3-anhydro-6-hydroxy-opsiobolin A resulted in the great enhancement of anti-microbial and anti-tumor activity assays (**Fig. 3**)^[62].

Fig. 3. Structures of compounds in Table 3, opiobolin with hydroxyl group at C-6.

In the activity assay of three compounds and etoposide against five human tumor cell lines, 6-epi-opsiobolin A, 3-anhydro-6-epi-opsiobolin A exhibited significant cytotoxic activity, have IC_{50} values in the range of $1\mu g/ml$, while opiobolin I was

less active with IC_{50} values more than $10\mu g/ml$, since the first two compounds have no significant difference in the activity, the 3-OH of 6-epi-opsiobolin A seemed to have little effect on the cytotoxic (**Fig. 4**)^[63].

Fig. 4 Structures of three compounds.

Indeed, according to convert ophiobolin A to its analogs under different conditions and compare their anticancer activities to cell lines, these data shows that the C5, C21-dicarbonyl functionality as critical for anticancer activity in this family of natural products. In addition, change or elimina-

tion the C5, C21-dicarbonyl functionality result in a significant drop in activity, such as 21,21-O-dihydrophiobolin A^[62] and ophiobolin I (Fig. 4)^[63]. In contrast, changes in the C15-C25 fragment, such as ophiobolin K, are tolerated (Fig. 5)^[22,64].

Fig. 5. Ophiobolin A and analogs. C5, C21-dicarbonyl and C15-C25 fragment.

Also compounds incorporating modifications of the C18, C19-alkene, have retained a significant portion of the antiproliferative potency, so the C15-C25 fragment and C18, C19-alkene, have little to do with their activities^[64-65].

Assayed on punctured detached leaves of several grass and dicotyledon weeds of

ophiobolin A, 6-epi-Ophiobolin A, 3-anhydro-6-epi-ophiobolin A and ophiobolin I, ophiobolin A proved to be on average more phytotoxic as compared to the other related compounds, some structural features appear to be important for the phytotoxicity, such as the hydroxy group at C-3, the stereochemistry at C-6, and the aldehyde group at C-7 (Fig. 6)^[57].

Fig. 6. Structural features appear to be important for the phytotoxicity.

Dereplication of some ophiobolin derivatives possessing different activity in combination with structural analysis allowed a correlation of the chemical structure and conformation with the extent of bioactivity, identifying the hydroxy group at C3 and an aldehyde at C21, as well as the A/B-*cis* ring structure, as indispensable for the strong activity of the ophiobolins^[3].

3.2. Configuration

Some literatures suggest configuration is

the cause of different activities of ophiobolin. According to compare three pairs of natural ophiobolin analogues for nematocidal activity in a *C. elegans* mobility assay (**Table 1**)^[15], stereoisomers with hydrogen at C-6 position in either β- (ophiobolin M, ophiobolin C and ophiobolin K) or α- (6-epiophiobolin M, 6-epiophiobolin C and 6-epiophiobolin K), in each case, ophiobolins with β-stereochemistry were found to be more potent (**Fig. 7**).

Fig. 7. Structures of compounds, ophiobolin with α and β- hydrogen.

3.3. The tricyclic 5-8-5 ring structure

As mentioned above, many ophiobolin family members have good bioactivities,

and their activity mainly depends on the unique functional groups or configurations based on their tricyclic 5-8-5 ring system. However, compounds with similar sester-

terpene skeleton usually show different activities, some of them are even less active, which suggest unique molecular structures is not the main factor of activity.

In the cytotoxicity against murine L5178Y lymphoma cells, five ophiobolin-type sesquiterpenoids were tested at a concentration of 10 µg/ml. As a result, none of the compounds reduced survival of cells by more than 10%-20% compared to control, this suggest that molecular structure may not be the main factor of ophiobolin activity (Fig. 8) [66-67].

In the determination of capacity to stimulate the seed germination of several orobanche species, ophiobolin A and several fusicoccin derivatives which have the similar sesquiterpene structure were tested in the concentration range of 10^{-4} - 10^{-7} M. The

results obtained showed that ophiobolin A(9), and the hexacetyl and pentacetyl isomers of 16-O-demethyl-de-tert-Pentenyl-fusicoccin(7,8) prepared by chemical modification of the fusicoccin show the highest stimulatory effect, while the fusicoccin(1) and other fusicoccin derivatives(2-6) appeared to be practically inactive (Fig. 9) [45, 68]. As shown in figure 9, with the same 5-8-5 ring system, compound 1-4 had the low stimulatory effect, while compound 7-8 and ophiobolin A showed the highest stimulatory effect. In addition, compound 5-6, which had the tricyclic 6-8-5 ring, also showed low stimulatory effect like compound 1-4. On this basis, it can be inferred there is no special relationship between bioactivity and the tricyclic 5-8-5 ring structure.

Fig. 8. Five ophiobolin-type sesquiterpenoids

Fig. 9. Structures of fusicoccin(1), some of its derivatives(2-6), fusicoccin deacetyl aglycone derivatives(7-8), ophiobolin A(9).

As mentioned above, there is no special relationship between bioactivity and the tricyclic 5-8-5 ring structure, so the functional group and configuration may be the causes of different activities of ophiobolin. As shown in **Fig 10**, ophiobolin and 6-epi-ophiobolin of each group (mentioned in Part 2.4) have the same planar

structures and functional groups, so the different configuration connected to C-6 may be the causes of different activities. Therefore, we mainly studied whether there were other factors that caused the activity difference of ophiobolin and 6-epi-ophiobolin.

Fig. 10. Structures of compounds of each group in Table 4

4. Structure-bioactivity relationship of ophiobolins and 6-epi ophiobolins

According to our study, we chose Ophiobolin O(1), K(3) and 6-epi-ophiobolin O (2), K (4) as objects which were isolated from one endophytic fungus-*Aspergillus sp*^[69], the molecular formulas were suggested as $C_{27}H_{42}O_4$ and $C_{25}H_{36}O_3$ using HRFABMS and ^{13}C NMR. (1) and (2), (3) and (4) were identical with the same planar structures and functional groups, while the bioactivity of them to mouse leukemia cell line (P388) also followed this role, showed

bioactivity to mouse leukemia cell line (P388) with MIC values of 4.7, 9.3, 13.3 and 24.9 μM , respectively, which indicated that there should have a difference. 1H and ^{13}C NMR spectra of them were analyzed in detail, as well as the 2-D spectra of COSY, TOCSY, HMBC and HSQC. During the absolute configuration elucidation, a big difference was found in the position C-7 and the hydrogen atom configuration connected to C-6. The hydrogen atom connected to C-6 in (1) was in β side, but in (2) was α side. In the study of (3) and (4), it showed same result (**Fig. 11**).

Fig. 11. The stereochemistry of ophiobolins and 6-epi-ophiobolin

Since the configuration connected to C-6 has been mentioned as the causes of different activities, we mainly studied the structures of C-7, including the calculation of bond lengths, bond orders and the molecular energy, the steric difference of the isomers was also discussed as well as the impact on the biological activity.

Coincidentally, these eight pairs of compounds in **Fig. 10** can be divided into two parts according to the structure at C-7, one part were A, C, K, M and 3-anhydro-6-hydroxy-ophiobolin A, the other were L, O and H (**Fig. 12**). So, the ophiobolin O and K which could cover these two structures and had good representativeness.

Structures in C-7(1)

Structures in C-7(2)

Fig. 12. Compounds with different structures in C-7

4.1. Theoretical study details

The theoretical calculations were carried out by using density functional theory (DFT) method implemented in the Gaussian 09 program^[70], and the hybrid exchange correlation functional B3LYP^[71-72] was employed. For C, H and O, all electrons were explicitly considered within the 6-31+G(d, p) basis set. All the geometrical structures were fully optimized, and vibrational frequency analyses were calculated to confirm that they are stable minima on the potential energy surface. Natural popula-

tion analyses (NPA) were carried out using the natural bond orbital (NBO) method^[73] implemented in NBO 5.0 program^[74]. By frequency analysis, all optimized geometries had no imaginary frequency and correspond to the lowest point of the reaction potential energy surface. In order to distinguish all atoms, all the related atoms were noted (**Fig. 13**). Through the optimization results, the stable configurations energy was accessed. All the optimized geometries bond length and bond order were listed (**Table 4, 5**).

Fig. 13. Optimized structures of compounds 1-4

Table 4. The calculation of bond length and bond order of compound 1 and 2.

Position	BondLength /nm		Bond order		Position	Bond Length /nm		Bond order	
	1	2	1	2		1	2	1	2
C(7)-H(40)	0.109 7	0.110 4	0.881 0	0.852 5	C(15)-H(49)	0.1092	0.1093	0.8998	0.9101
C(6)-C(7)	0.151 9	0.150 2	0.994 2	0.991 2	C(16)-C(29)	0.1536	0.1530	1.0095	1.0149
C(6)-C(13)	0.133 8	0.133 4	1.871 4	1.876 4	C(16)-O(22)	0.1464	0.1471	0.9066	0.9041
C(13)-C(12)	0.150 9	0.151 6	1.022 1	1.022 3	C(14)-O(21)	0.1468	0.1440	0.8724	0.9217
C(12)-C(11)	0.155 6	0.156 1	0.981 2	0.991 7	C(20)-O(21)	0.1446	0.1498	0.9072	0.8602
C(11)-C(10)	0.157 1	0.158 4	0.970 1	0.968 5	C(6)-C(20)	0.1512	0.1528	0.9908	0.9867
C(10)-C(9)	0.156 6	0.155 6	0.984 2	0.986 7	C(20)-O(27)	0.1443	0.1426	0.9035	0.9389
C(9)-C(8)	0.154 6	0.153 5	1.005 8	1.001 7	C(10)-C(17)	0.1560	0.1560	0.9784	0.9783
C(8)-C(7)	0.158 3	0.153 0	0.964 4	0.982 4	C(17)-C(18)	0.1545	0.1534	1.0092	1.0106
C(8)-H(41)	0.109 4	0.109 4	0.904 3	0.882 0	C(18)-C(19)	0.1563	0.1554	0.9908	0.9949
C(13)-H(47)	0.109 1	0.109 1	0.907 8	0.910 5	C(19)-C(11)	0.1585	0.1605	0.9763	0.9733

C(12)-H(45)	0.109 6	0.109 8	0.908 5	0.910 5	C(19)-C(1)	0.1565	0.1565	0.9784	0.9792
C(12)-H(46)	0.109 7	0.109 8	0.906 3	0.892 7	C(17)-H(50)	0.1099	0.1100	0.9134	0.9142
C(11)-H(44)	0.109 9	0.109 8	0.894 9	0.901 2	C(17)-H(51)	0.1097	0.1097	0.9168	0.9163
C(10)-C(30)	0.154 6	0.154 6	0.995 6	0.996 2	C(18)-H(52)	0.1096	0.1095	0.9146	0.9134
C(9)-H(42)	0.109 7	0.109 8	0.901 5	0.905 5	C(18)-H(53)	0.1097	0.1096	0.9179	0.9152
C(9)-H(43)	0.109 7	0.110 4	0.901 8	0.914 9	C(19)-H(54)	0.1099	0.1099	0.9014	0.9007
C(7)-C(14)	0.156 9	0.152 9	0.956 6	0.958 8	C(1)-C(31)	0.1546	0.1549	0.9980	0.9970
C(14)-C(15)	0.152 3	0.152 1	0.987 4	0.991 3	C(1)-C(23)	0.1515	0.1515	1.0142	1.0137
C(15)-C(16)	0.153 1	0.157 3	0.982 7	0.970 6	C(23)-C(24)	0.1349	0.1349	1.8252	1.8248
C(16)-C(8)	0.159 7	0.160 7	0.947 8	0.947 9	C(24)-C(2)	0.1456	0.1456	1.1301	1.1303
C(14)-O(25)	0.143 0	0.146 2	0.930 2	0.871 5	C(2)-C(4)	0.1355	0.1355	1.7714	1.7712
C(15)-H(48)	0.109 3	0.109 2	0.879 2	0.884 4	C(4)-C(3)	0.1511	0.1511	1.0297	1.0298

Table 5. The calculation of bond length and bond order of compound 3 and 4.

Position	Bond Length /nm		Bond order		Position	BondLength/nm		Bond order	
	3	4	3	4		3	4	3	4
C(7)-H(37)	0.110 7	0.110 6	0.857 3	0.842 8	C(15)-H(45)	0.1096	0.1094	0.8715	0.8753
C(6)-C(7)	0.150 8	0.151 4	0.997 7	0.997 8	C(15)-H(46)	0.1096	0.1098	0.8870	0.8840
C(6)-C(13)	0.135 5	0.135 2	1.748 9	1.794 1	C(16)-C(28)	0.1539	0.1528	1.0061	1.0101
C(13)-C(12)	0.150 4	0.151 0	1.044 6	1.029 8	C(16)-O(24)	0.1463	0.1474	0.9053	0.8934
C(12)-C(11)	0.154 1	0.156 4	0.995 1	0.978 0	C(6)-C(20)	0.1484	0.1495	1.0764	1.0499
C(11)-C(10)	0.156 7	0.157 0	0.969 5	0.970 0	C(20)-O(22)	0.1242	0.1243	1.7883	1.7927

C(10)-C(9)	0.157 2	0.155 4	0.982 7	0.988 1	C(10)-C(17)	0.1558	0.1555	0.9795	0.9780
C(9)-C(8)	0.155 9	0.154 9	1.003 5	0.994 6	C(17)-C(18)	0.1539	0.1544	1.0086	1.0088
C(8)-C(7)	0.157 0	0.155 6	0.960 2	0.981 1	C(18)-C(19)	0.1558	0.1563	0.9936	0.9906
C(8)-H(38)	0.109 9	0.109 6	0.898 8	0.889 9	C(19)-C(11)	0.1597	0.1592	0.9732	0.9774
C(13)-H(44)	0.109 1	0.109 1	0.889 5	0.887 3	C(19)-C(1)	0.1558	0.1554	0.9889	0.9898
C(12)-H(42)	0.110 4	0.109 3	0.864 8	0.905 6	C(17)-H(47)	0.1095	0.1100	0.8905	0.9151
C(12)-H(43)	0.109 5	0.109 5	0.899 9	0.899 3	C(17)-H(48)	0.1098	0.1097	0.9189	0.9152
C(11)-H(41)	0.108 9	0.109 9	0.895 8	0.898 9	C(18)-H(49)	0.1095	0.1094	0.9147	0.9141
C(10)-C(21)	0.105 1	0.154 7	0.995 6	0.994 0	C(18)-H(50)	0.1095	0.1096	0.9158	0.9146
C(9)-H(39)	0.110 1	0.109 9	0.911 4	0.909 8	C(19)-H(51)	0.1098	0.1099	0.9002	0.8999
C(9)-H(40)	0.109 8	0.110 2	0.906 9	0.914 6	C(1)-C(27)	0.1552	0.1548	0.9953	0.9991
C(7)-C(14)	0.153 5	0.154 4	0.964 0	0.955 9	C(1)-C(25)	0.1520	0.1521	1.0202	1.0195
C(14)-C(15)	0.152 4	0.151 9	0.990 3	0.994 8	C(25)-C(26)	0.1349	0.1349	1.8199	1.8188
C(15)-C(16)	0.154 1	0.153 5	0.983 8	0.984 9	C(26)-C(2)	0.1457	0.1457	1.1275	1.1285
C(16)-C(8)	0.158 3	0.157 1	0.954 4	0.961 6	C(2)-C(4)	0.1355	0.1355	1.7728	1.7717
C(14)-O(23)	0.123 6	0.123 5	1.838 2	1.845 8	C(4)-C(3)	0.1511	0.1511	1.0296	1.0298

4.2. Analysis of optimization results

Table 6 shows the bond length of C(7)-H(40) in 1 (0.1097 nm) was shorter than 2 (0.1104 nm), which confirmed that the bond energy of C(7)-H(40) in 1 was higher than 2, and 1 was better than 2 in the thermodynamic stability of this chem-

ical bond. Also the bond order of C(7)-H(40) in 1 (0.8810) was bigger than 2 (0.8525), suggested that the bond energy of C(7)-H(40) in 1 was higher than 2. It also supported that 1 was better than 2 in the thermodynamic stability. The overall energy of the molecular of 1(-1354.6121) was lower than 2(-1354.5984), It was con-

firmed that the chemical stability of 1 was higher than 2. In conclusion, 1 was superior to 2 both from the thermodynamic stability and chemical stability, and it also indicated that the steric hindrance of 1 was less than 2.

Using the same method, the bond length of C(7)-H(37) in 3 and 4 were basically the same, while the bond order of C(7)-H(37) of 3 was still bigger than 2, suggested that the bond energy of C(7)-H(37) in 3 was higher than 4, and had higher thermodynamic stability in this position. Also, the bigger bond order of C(7)-H(37) of 3

(0.8573) suggested that the bond energy of C(7)-H(37) of 3 was higher than 4. It also indicated that the thermodynamic stability in this bond of 3 was better than 4. The overall energy of the molecular indicated that the overall molecular chemical stability of 4 was better than 3, it was interesting result that 3 had more thermodynamic stability than 4 on the key structure of C(7), but the chemical stability of the overall molecular of 3 was weaker than 4. It indicated that the whole steric hindrance of 3 was higher than 4, but in the position C(7)-H(37), it showed opposite result that the steric hindrance of 3 was less than 4.

Table 6. Theoretical calculation of the basic parameters of ophiobolin.

Compound	O(1)	O(2)	名称	K(3)	K(4)
Bond Length of (7)-H(40)(nm)	0.1097	0.1104	Bond Length of (7)-H(37)(nm)	0.1107	0.1106
Bond order of C(7)-H(40)	0.8810	0.8525	Bond order of C(7)-H(37)	0.8573	0.8428
Overall energy (hartree)	-1354.6121	-1354.5984	Overall energy (hartree)	-1199.6120	-1199.6223

The optimized whole molecular energy analysis suggested opposite results in molecular integral steric hindrance of compounds 1, 2 and 3, 4. It was hard to explain the bioactivity regularities of ophiobolins using the whole molecular steric hindrance method. But in the position C(7), all the analysis showed the same pattern, both lower steric hindrance and strong biological activity. It determined that the C(7) was one bioactive functional group in the ophiobolins. The steric hindrance size directly affected the biological activity of compounds. The compounds attacking the target should be much easier within smaller steric hindrance size and showed higher bioactivity.

It was also easily understood that the

overall steric hindrance of 3 and 4 didn't follow this rule. Not all the functional groups in the molecule 3 and 4 were key bioactive functional groups. It suggested that the steric hindrance determination of partial functional groups could be used as one criterion to find bioactive functional groups of target compounds.

5. Conclusions

According to a systematic review of the ophiobolin family members that have been found, give a brief introduction including resource, structure, bioactivity, The mode of action and the possible reasons that cause the different bioactivity. Taking ophiobolin O, K and 6-epi-ophiobolin O, K as an example, which had the same planar

structures but different activity, according to calculate the bond lengths and bond orders, together with whole molecular energy using density functional theory at B3LYP/6-31+G(d, p) level, It was found that the steric hindrance of active functional groups was the key factor of bioactivity. The functional groups with low steric hindrance could be easier attacking the target, and thereby enhance the compounds bioactivity.

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