Dissertation Title	Secondary Metabolites of Selected Helicosporous Tubeufiaceae
Author	Lijuan Zhang
Degree	Doctor of Philosophy (Biological Science)
Advisor	Assistant Professor Mahamarakkalage Mary Ruvishika Shehali Jayawardena, Ph. D.
Co-Advisor	Professor Yong-Zhong Lu, Ph. D.

ABSTRACT

Helicosporous hyphomycetes is a group of saprophytic fungi, with occasional endophytic forms. Its characteristic feature is the presence of helicoid conidia, which can coil at least 180° on a two-dimensional or three-dimensional plane. Despite their distinctive morphology and strong biosynthetic potential—yielding alkaloids, glycolipids, polyketides, and terpenoids with antibacterial, antitumor, and antidiabetic activities—chemical studies remain limited. In our prior work, we accumulated an extensive Tubeufiaceae strain collection and, via small-scale solid-state fermentations, generated crude extracts for chemical and cytotoxicity screening. Five strains—*Neohelicosporium guangxiense* (SLGY-5), *N. griseum* (GYSLGY-1, SLGY-15), *Tubeufia longihelicospora* (WZS71), and *Helicosporium sexuale* (LZ15)—were prioritized for detailed metabolite and bioactivity studies.

From *Neohelicosporium guangxiense* SLGY-5, three *α*-tetralone derivatives (**C1**–**C3**) and one isocoumarin (**C4**) were isolated; notably, **C1** was obtained from natural sources for the first time. From *N. griseum* GYSLGY-1, two new polyketides—neogrisphenol A (**C5**) and neogrisphenol B (**C6**)—one new isochroman-1-one, (*S*)-6-hydroxy-7-methoxy-3,5-dimethylisochroman-1-one (**C7**), and four known compounds (**C8**–**C11**) were characterized. From *N. griseum* SLGY-15, seven polyketides (**C12**–**C18**) and one steroid (**C19**) were obtained. From *Tubeufia longihelicospor*a WZS71, two dimeric naphtho-*γ*-pyrones (**C20**–**C21**), two dimeric coumarins (**C22**–**C23**), and one alkaloid (**C24**) were isolated. From *Helicosporium sexuale* LZ15, seven new dibenzo-*α*-pyrone derivatives—Helicolide A (**C25**), Helicohydrins B–D (**C26**–**C28**), and Helicochlorins E–G (**C29**–**C31**)—together with eight known congeners (**C32**–**C39**) were obtained. Structures were elucidated by 1D/2D NMR,

mass spectrometry, NOE analysis, single-crystal X-ray diffraction, and ECD, supported by quantum-chemical calculations.

Cytotoxicity testing revealed that neogrisphenol A (**C5**) showed potent activity against A2780, PC-3, and MDA-MB-231 cells (IC_{50} = 3.20, 10.68, and 16.30 μM, respectively), exceeding the potency of cisplatin (CDDP) in A2780 under the same conditions. Neogrisphenol B (**C6**) was also active against A2780 (IC_{50} = 10.13 μM). *In vitro* mechanistic assays indicated that **C5** suppressed A2780 proliferation, induced apoptosis, and caused S-phase cell-cycle arrest in a concentration-dependent manner. Compounds **C12**, **C14**, and **C15** exhibited cytotoxicity toward HeLa cells (IC_{50} = 30.8, 13.7, and 14.1 μM) and A549 cells (IC_{50} = 24.7, 7.4, and 10.3 μM), respectively. Compound **C36** exhibited potent activity in HepG2/HeLa (IC_{50} both < 20 μM), indicating selectivity toward these two cell lines; compound **C38** showed moderate activity in A549/HeLa (16.55–26.49 μM), also displaying selectivity; compound **C37** was moderate and selective toward A2780 (25.73 μM); and compound **C30** showed moderate activity only in A549 (38.81 μM). Compound **C32** exhibited inhibitory activity across multiple cell lines with IC_{50} values of 22.49–85.14 μM, indicating broad-spectrum activity.

Antimicrobial testing showed that compounds **C5–C6** and **C10** were active against *Bacillus subtilis*, *Clostridium perfringens*, and *Staphylococcus aureus* (MIC 16–31 μg/mL). Compounds **C12–C15** displayed moderate activity against *Pseudomonas aeruginosa*, with **C13** also showing weak activity against *S. aureus*. Compounds **C20** and **C21** were active against *P. aeruginosa*, with MIC 62.0 μg/mL and MBC 248 μg/mL. In addition, compound **C9** showed antifungal activity against *Sclerotinia sclerotiorum* and *Phytophthora nicotianae* var. nicotianae, with respective IC_{50} values of 88.14 ± 2.21 μg/mL and 52.36 ± 1.38 μg/mL.

Overall, this work expands the chemical space of helicosporous hyphomycetes and yields promising lead scaffolds; coupling genome mining and metabolomics with SAR/MoA studies around **C5**, and leveraging scale-up fermentation and semisynthesis for supply and derivatization, will accelerate the discovery of antibacterial and anticancer candidates.

Keywords: Helicosporous Hyphomycetes, Tubeufiaceae, *Neohelicosporium*, *Tubeufia*, *Helicosporium*, Secondary Metabolites, Polyketides, *A*-Tetralone, Dibenzo-*A*-Pyrone, Antifungal, Antibacterial, Cytotoxicity