

# Small Molecule S4286 Targets Mitochondria and Induces Autophagy to Control *Sclerotinia Sclerotiorum*

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## Abstract

Plant pathogenic fungi such as *Sclerotinia sclerotiorum*, *Botrytis cinerea*, and *Magnaporthe oryzae* pose severe threats to crop yields worldwide. Here, we identified a novel small molecule compound, S4286, from a chemical library screening, and systematically evaluated its antifungal activities against *S. sclerotiorum* and other agriculturally significant fungal pathogens. In vitro assays demonstrated that S4286 exhibited potent antifungal activity, effectively inhibiting growth of both sensitive and carbendazim-resistant strains of *S. sclerotiorum* at low concentrations (the average EC<sub>50</sub> was 0.032 µg/mL). Detached leaf assays further confirmed that S4286 significantly reduced lesion areas on rapeseed leaves within a concentration range of 0.1–10 µg/mL, showing superior efficacy compared to the conventional fungicide carbendazim. Moreover, broad-spectrum antifungal activity was observed against other tested fungal pathogens. Microscopic examination revealed morphological abnormalities in fungal hyphae, including tip swelling, distortion, mitochondrial structural disruption, and autophagic structures, indicating induction of autophagy. Consistently, increased reactive oxygen species (ROS) accumulation and decreased mitochondrial membrane potential suggested the occurrence of oxidative stress and mitochondrial dysfunction. Transcriptome analysis revealed that genes related to oxidative phosphorylation pathways were broadly downregulated upon S4286 treatment, alongside significant modulation of genes involved in energy metabolism and cell-wall biosynthesis. qPCR assays confirmed upregulated expression of autophagy-related (ATG) genes. Collectively, these findings highlight the remarkable potential of small molecule compound S4286 as a novel antifungal agent with a unique mode of action involving mitochondrial dysfunction, oxidative stress induction, and autophagy. Future field efficacy trials and crop safety evaluations will be essential to validate its agricultural application prospects.

## Keywords

*Sclerotinia Sclerotiorum*, Mitochondrial Dysfunction, Oxidative Stress, Autophagy, Fungicide Resistance