

脱氢枞基 1, 2, 4-三唑类衍生物的合成及其抗真菌活性研究

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摘要:【目的】针对于农作物病疫泛滥,设计开发环境相容性好、细胞毒性低的松香基药剂分子并初步探究其作用机制。【方法】通过活性亚结构拼接原理将脱氢枞酸、1,2,4-三唑环通过席夫碱基团有机组装;采用菌丝生长速率法测试目标化合物对苹果腐烂、黄瓜炭疽、小麦赤霉、油菜菌核、和小麦全蚀 5 种植物真菌的抗真菌活性;利用扫描、透射电镜和激光共聚焦显微镜观察化合物 **3f** 对菌丝体超微结构的影响;通过生理生化指标测试和分子对接技术初步探究化合物 **3f** 对苹果腐烂真菌作用机制。【结果】设计合成 22 个未见文献报道的脱氢枞基 1,2,4-三唑席夫碱类衍生物;体外活性测试结果表明:化合物 **3f** 对苹果腐烂真菌具有较好的抗真菌活性 ($EC_{50} = 0.537 \mu\text{g/mL}$),显著优于阳性对照氟康唑 ($EC_{50} = 4.689 \mu\text{g/mL}$);体内活性测试结果表明:在 25-100 $\mu\text{g/mL}$ 浓度下,化合物 **3f** 对苹果树枝具有较好的保护作用 (61.57%-92.16%),略低于氟康唑 (85.17%-100%);菌丝超微结构观测结果显示:化合物 **3f** 阻碍了菌丝体的生长并严重破坏苹果腐烂真菌的细胞膜、线粒体等细胞结构;激光共聚焦显微镜染色结果进一步表明:化合物 **3f** 处理苹果腐烂真菌能显著引起活性氧累计,线粒体膜电位发生变化;生理生化测试结果表明:化合物 **3f** 能显著降低 CYP51、SOD 和 CAT 的活性 (分别为 59.70%、76.9%和 67.86%),致使苹果腐烂真菌细胞膜通透性发生改变、活性氧大量累计;分子对接结果表明:化合物 **3f** 和 CYP51 (-11.18 kcal/mol)、SOD (-9.25 kcal/mol) 和 CAT (-8.79 kcal/mol) 之间存在较强的结合能,其中化合物 **3f** 与脯氨酸、缬氨酸成疏水作用,与组氨酸、精氨酸形成氢键,进而导致 CYP51、SOD 和 CAT 的活性降低。【结论】基于羧基改性制备脱氢枞基 1,2,4-三唑类衍生物可实现对苹果腐烂真菌的有效防治,其中 CYP51、SOD 和 CAT 是该类化合物的潜在作用靶点。

Synthesis and Antifungal Activity of Dehydroabienyl-1,2,4-Triazole Derivatives

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Abstract: 【Objective】To design and develop rosin based drug molecules with good environmental compatibility and low cytotoxicity for the spread of crop diseases and epidemics, and preliminarily explore their mode of action of target compound. 【Method】Dehydroabietic acid and 1,2,4-triazole rings were organically assembled through Schiff base groups using the principle of active substructure splicing. The mycelial growth rate method was used to measure the antifungal activity of the target compound against five plant fungi named *Valsa*

mali, *Colletotrichum orbiculare*, *Fusarium graminearum*, *Sclerotinia sclerotiorum* and *Gaeumannomyces graminis*. The ultrastructure of mycelium was observed via scanning electron microscope (SEM), transmission electron microscope (TEM) and confocal laser scanning microscope (CLSM). The mode of action of compound **3f** on *V. mali* was explored through the measurement of physiological and biochemical indicators and molecular docking.

【Result】 22 novel dehydroabietyl-1,2,4-triazole-schiff based derivatives were designed and synthesized. The *in vitro* activity measurement showed that compound **3f** had good antifungal activity against *V. mali* ($EC_{50} = 0.537 \mu\text{g/mL}$), significantly better than the positive control fluconazole ($EC_{50} = 4.689 \mu\text{g/mL}$). The *in vivo* antifungal activity results indicated that compound **3f** had a good protective effect on apple branches (61.57% -92.16%), slightly lower than fluconazole (85.17% -100%). The observation results of mycelium ultrastructure showed that compound **3f** hindered the growth of mycelium and severely damaged the cell membrane, mitochondria, and other cellular structures of *V. mali*. The staining results further indicated that the mycelia treated with compound **3f** produced the accumulation of reactive oxygen species (ROS) and changes in mitochondrial membrane potential. The results of physiological and biochemical tests showed that compound **3f** significantly reduced the activities of CYP51, SOD, and CAT (59.70%, 76.9%, and 67.86%, respectively), resulting in the change of permeability of the cell membrane, and the accumulation of ROS. The molecular docking results indicated that there was a strong binding energy between compound **3f** and CYP51 (-11.18 kcal/mol), SOD (-9.25 kcal/mol), and CAT (-8.79 kcal/mol). Compound **3f** formed hydrophobic interactions with proline and valine, forming hydrogen bonds with histidine and arginine, leading to a decrease in the activity of CYP51, SOD, and CAT. **【Conclusion】** The preparation of dehydroabietyl-1,2,4-triazole derivatives based on carboxyl modification could achieve effective prevention and control of *V. mali*, and CYP51, SOD and CAT were potential mode of action for these compounds.