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INSIGHTS FROM *in vitro* AND *in vivo* STUDIES OF 4-OXO-4H-1-BENZOPYRAN-3-CARBALDEHYDE AGAINST *Aspergillus* species

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Abstract

Aspergillus spp. is the culprit behind various infectious diseases that contribute significantly to the yearly global death toll. Millions of cases, including chronic pulmonary aspergillosis, invasive aspergillosis, and allergic bronchopulmonary aspergillosis, are reported worldwide annually. Its resistance is growing to conventional antifungals, especially azoles, highlights the urgent need for new and effective therapeutic alternatives. In this manuscript, the anti-*Aspergillus* activity of 4-oxo-4H-1-benzopyran-3-carbaldehyde was studied. Kirby-Bauer test, Broth Microdilution Assay, Spore Germination Inhibition Assay, and Scanning Electron Microscopy technique were employed for the *in vitro* study. The acute toxicity and survival rate were studied on Wistar rats. The compound exhibited a broad spectrum of antifungal activity, with minimum inhibitory concentrations (MICs) ranging from 0.977 to 125 mg/L. Notably, it showed strong inhibition against multiple *A. fumigatus* strains (MIC: 3.9–15.6 mg/L) and *A. niger* ITCC 5405 (MIC: 0.977 mg/L), while strain-dependent resistance was observed in *A. flavus* and *A. niger* PGIMS Rohtak (MIC: 31.25–125 mg/L). SEM analysis of *A. fumigatus* PGIMS Rohtak revealed severe morphological disruptions, indicating membrane integrity loss and cellular damage. Additionally, acute toxicity and survival studies in Wistar rats demonstrated the compound's non-toxic nature and significant therapeutic potential, with treated groups showing a notable improvement in survival rates ($P = 0.001$). Overall, 4-oxo-4H-1-benzopyran-3-carbaldehyde exhibited promising antifungal activity, membrane-disrupting effects, and safe toxicity profiles, making it a potential candidate for managing drug-resistant fungal infections. Further studies on resistance mechanisms and structural modifications could enhance its efficacy for clinical applications.

Key words: antifungal, *Aspergillus*, benzopyran derivatives, *in vitro*, *in vivo*, 4-oxo-4H-1-benzopyran-3-carbaldehyde

Received: July, 2024; Revised final: April, 2025; Accepted: May, 2025; Published in final edited form: May, 2026

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