

RESEARCH ARTICLE

Screening novel antiviral compounds to treat *Clostridioides difficile* infectionsBrice J. Stoltz^{1,2}, Ahmed A. Abouelkhair^{1,2}, Mohamed N. Seleem^{1,2*}

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Abstract

Clostridioides difficile is a major cause of nosocomial infections, often associated with individuals who have gut dysbiosis from previous antibiotic therapies. *C. difficile* infections (CDI) have a high recurrence rate and impose significant financial and mortality burdens on the healthcare system. Therefore, novel anti-*C. difficile* drugs are urgently needed to treat and reduce the severity and recurrence of infection. In this study, we screened a library of 618 antiviral drugs to identify a potential candidate for repurposing as novel anti-*C. difficile* therapeutics. Following our preliminary screening, we identified 9 novel compounds that inhibited *C. difficile* at a concentration of 16 μ M or lower. Among these, 4 antiviral compounds demonstrated the most potent anti-*C. difficile* activity against a panel of 15 *C. difficile* isolates, with minimum inhibitory concentrations (MICs) comparable to the drug of choice, vancomycin. These include rottlerin (MIC₅₀ = 0.25 μ g/mL), α -mangostin (MIC₅₀ = 1 μ g/mL), dryocrassin ABBA (MIC₅₀ = 1 μ g/mL), and obelazimod (MIC₅₀ = 4 μ g/mL). All exhibited minimal to no activity against representative members of the human gut microbiota. Interestingly, α -mangostin, a natural xanthone derived from the mangosteen fruit, exhibited strong bactericidal action, clearing a high inoculum of *C. difficile* in less than an hour. All other drugs exhibited bacteriostatic activity. Given their characteristics, these compounds show great promise as novel treatments for CDI.

Introduction

Healthcare-associated infections are one of the greatest burdens on healthcare systems. Most bacteria responsible for a part of this burden are associated with antimicrobial resistance, with one notable exception: *C. difficile*. Despite lacking a significant antimicrobial resistance profile, *C. difficile* is still considered an urgent threat and one of the deadliest bacterial infections responsible for approximately 30,000 deaths annually, primarily affecting older populations [1–5]. The incidence of *C. difficile* infections (CDI) in the United States has increased over the last two decades and has remained relatively stable from 2021 to 2022, with the COVID-19 pandemic seeing a drop in cases due to improved strategies preventing the spread of

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Table of Contents Screening The Novel

1. Understanding the eBook Screening The Novel
 - The Rise of Digital Reading Screening The Novel
 - Advantages of eBooks Over Traditional Books
2. Identifying Screening The Novel
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Screening The Novel
 - User-Friendly Interface
4. Exploring eBook Recommendations from Screening The Novel
 - Personalized Recommendations
 - Screening The Novel User Reviews and Ratings
 - Screening The Novel and Bestseller Lists

5. Accessing Screening The Novel Free and Paid eBooks
 - Screening The Novel Public Domain eBooks
 - Screening The Novel eBook Subscription Services
 - Screening The Novel Budget-Friendly Options
6. Navigating Screening The Novel eBook Formats
 - ePub, PDF, MOBI, and More
 - Screening The Novel Compatibility with Devices
 - Screening The Novel Enhanced eBook Features
7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Screening The Novel
 - Highlighting and Note-Taking Screening The Novel
 - Interactive Elements Screening The Novel
8. Staying Engaged with Screening The Novel
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Screening The Novel
9. Balancing eBooks and Physical Books Screening The Novel
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Screening The Novel
10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
11. Cultivating a Reading Routine Screening The Novel
 - Setting Reading Goals Screening The Novel
 - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Screening The Novel
 - Fact-Checking eBook Content of Screening The Novel
 - Distinguishing Credible Sources
13. Promoting Lifelong Learning

- Utilizing eBooks for Skill Development
- Exploring Educational eBooks

14. Embracing eBook Trends

- Integration of Multimedia Elements
- Interactive and Gamified eBooks

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