

Protease-Activated Trojan Horses: A New Route to Defeat *Acinetobacter baumannii*

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Multidrug-resistant (MDR) *Acinetobacter baumannii* is a major global health concern, responsible for severe hospital-acquired infections such as pneumonia, septicemia, and sepsis. Its ability to persist under adverse conditions and acquire antibiotic resistance mechanisms has led the World Health Organization to classify it as a **critical priority pathogen**, highlighting the urgent need for new antibacterial approaches that can overcome its impermeable outer membrane and active efflux systems.¹

One promising approach exploits bacterial iron uptake systems through siderophore-mediated transport, enabling antibiotic delivery directly into bacteria — the “Trojan horse” strategy. Antibiotic-siderophore conjugates derived from this concept are collectively known as sideromycins, which act as molecular vectors that utilize bacterial iron uptake mechanisms to introduce antibiotics across the cell envelope.²

In this context, we have developed a protease-activated siderophore–antibiotic conjugate designed to selectively target *A. baumannii*. The conjugate combines a simplified amonabactin analogue (AMB), with the protease-cleavable peptide linker WSPKYM, and norfloxacin (NFX) as antibiotic. Amonabactin was selected due to its catecholate-based structure and molecular simplicity, which allow recognition by outer membrane receptors shared among several Gram-negative bacteria, including *A. baumannii*.³⁻⁵

A model conjugate (WSPKYM – NFX) was first synthesized and incubated with a cell free extract of periplasmic proteases from *Aeromonas salmonicida* to validate the cleavage efficiency of the linker. HPLC/MS analysis confirmed successful cleavage of the WSPKYM linker, indicating an efficient protease recognition and antibiotic release mechanism.⁶

The conjugate (AMB – WSPKYM – NFX) was obtained through a hybrid synthetic route, combining solid-phase peptide synthesis for the preparation of the linker and the siderophore fragments with solution-phase reaction steps for the final coupling with norfloxacin.

This work represents the first report of the preparation of a protease-activated amonabactin-based conjugate designed for *A. baumannii*, to demonstrate the versatility of this chemical platform that combines siderophore-mediated internalization with enzyme activation. Such conjugates could serve as next-generation antibacterial agents capable of bypassing permeability barriers and targeting resistant Gram-negative pathogens.

[1] Gaddy, J. A.; Actis, L. A. *Front. Cell. Infect. Microbiol.* **2009**, 9, 17

[2] Ghosh, M.; Miller, P. A.; Möllmann, U.; Claypool, W. D. *et al. J. Med. Chem.* **2017**, 60, 4086–4093.

[3] Balado, M.; Lemos, M. L.; Osorio, C. R. *et al. ACS Chem. Biol.* **2015**, 10, 2850–2860.

[4] Rey-Varela, D.; Cisneros-Sureda, J.; Rodríguez, J.; Lemos, M. L. *et al. ACS Infect. Dis.* **2019**, 5, 1936–1951.

[5] Cisneros-Sureda, J.; Rey-Varela, D.; Lemos, M. L.; Osorio, C. R. *et al. J. Inorg. Biochem.* **2022**, 230, 111743.

[6] Boyce, J. H.; Muir, T. W. *J. Am. Chem. Soc.* **2020**, 142, 21310–21321.