# Esterase-induced release of a theranostic prodrug in lysosomes for improved therapeutic efficacy and lower systemic toxicity

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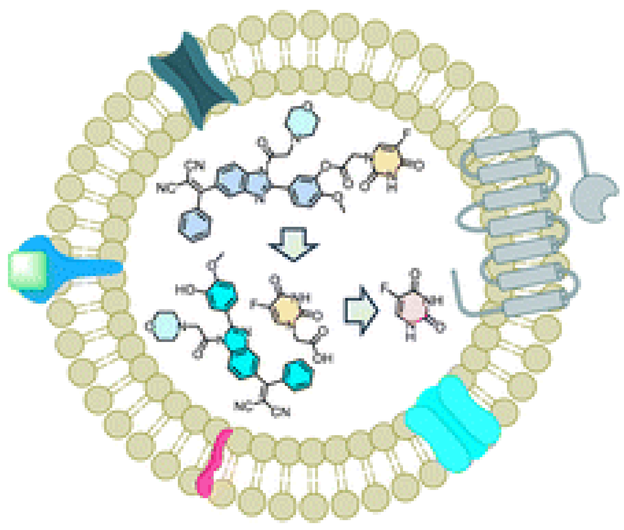
## What's the study about?

This work presents a lysosome-targeted theranostic prodrug of 5-fluorouracil (5-FU) that links 5-fluorouracil-1-acetic acid (FUA) to a benzimidazole fluorophore and a morpholine group via an esterase-cleavable ester. The design keeps the conjugate non-fluorescent and less active during circulation, then selectively activates inside lysosomes where intracellular esterases trigger bond cleavage. The aim is to achieve site-specific, sustained release of 5-FU within cancer cells while simultaneously switching on fluorescence to track drug activation—thereby improving efficacy and reducing off-target toxicity.



## How was the study conducted?

The team synthesized and fully characterized the prodrug and controls, then quantified esterase-induced cleavage using fluorescence turn-on and RP-HPLC to map release kinetics across pH conditions. Cellular uptake and lysosomal localization were visualized by confocal microscopy. Cytotoxicity was measured in cancer cell lines (e.g., U87 glioblastoma and SKOV-3 ovarian) and compared with a normal CHO cell line. Mechanistic studies used flow cytometry to evaluate cell-cycle effects and apoptosis. Functional relevance was further tested through anti-angiogenic assays on the CAM model and growth inhibition of 3D HeLa spheroids.

## Key findings

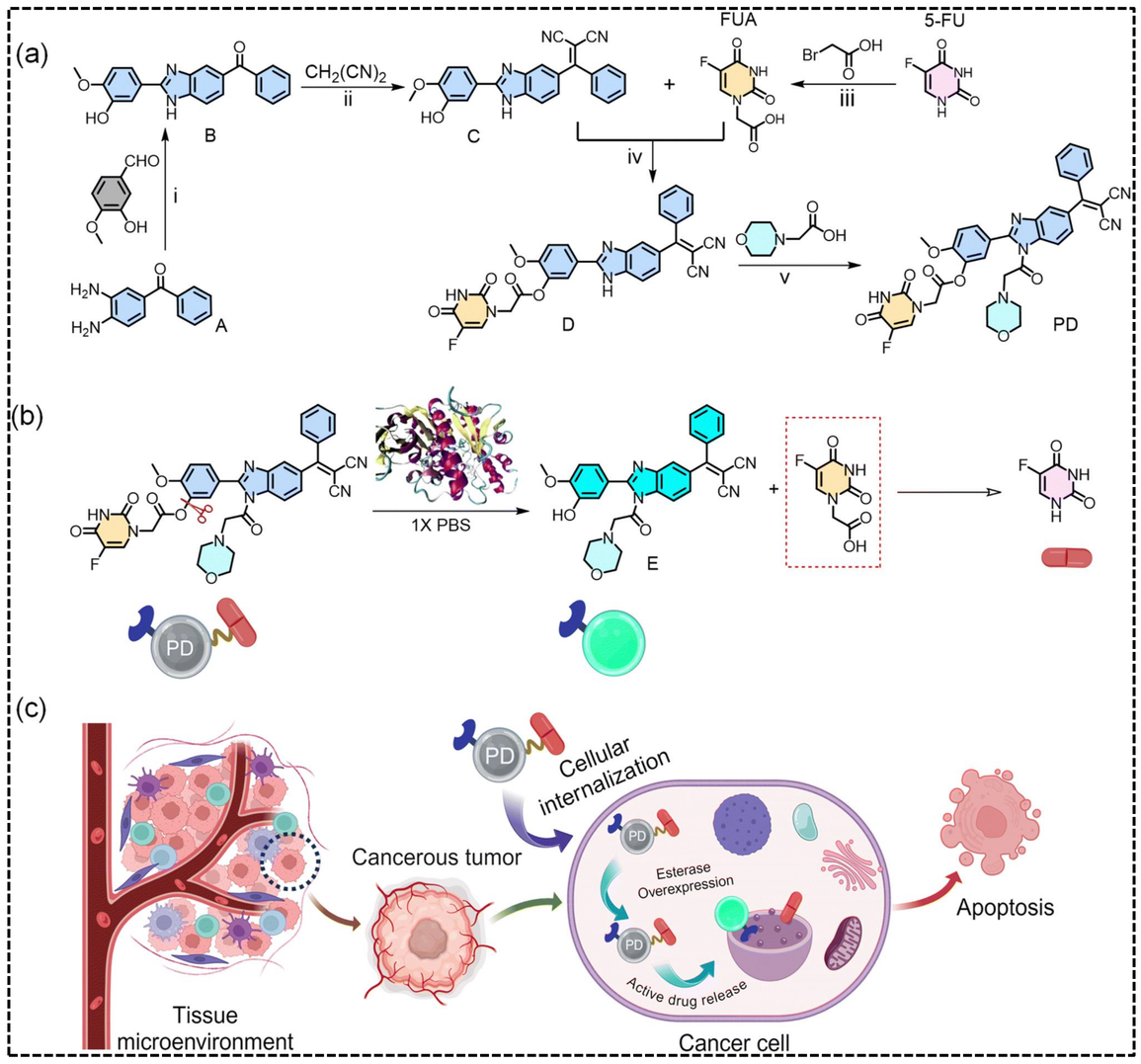
Esterase exposure converted the "OFF" prodrug to a fluorescent "ON" state, confirming triggerable cleavage and sustained release that approached a plateau around ~6 hours, with faster kinetics under acidic conditions consistent with lysosomes. The prodrug localized to lysosomes and showed greater potency than free 5-FU in cancer cells (e.g., $IC_{50} \approx 20.8$ μM in U87 and ≈ 36.9 μM in SKOV-3 versus ≈ 105 μM and ≈ 48 μM for 5-FU, respectively), while sparing normal CHO cells (≈ 95% viability at 50 μM). Mechanistically, treatment produced S-phase (± G2/M) arrest and both early and late apoptosis. Functionally, the prodrug exerted stronger anti-angiogenic effects and significantly reduced 3D spheroid size relative to controls.

## Novelty

Lysosome-targeted theranostic 5-FU prodrug with esterase-triggered, sustained release and fluorescence turn-on; dual imaging-plus-therapy in a single construct.

## Mechanism

Targeting the lysosome—a metabolic and degradative hub—enables concentrated intracytoplasmic release of 5-FU where it is most effective, which can increase tumor cell kill at lower systemic exposure. Because activation is fluorescence-tracked, clinicians gain a built-in readout of drug engagement, supporting dose optimization and response monitoring. Together, these properties point to a pathway for more effective and safer chemotherapy, with a translational trajectory that could leverage established knowledge of 5-FU.




- **Societal benefits:** Potentially fewer side effects, better quality of life, reduced hospital burden and costs; imaging-guided dosing can limit overtreatment—beneficial for resource-constrained settings.
- **SDG alignment:** SDG 3 (Good Health & Well-Being) via safer, more effective cancer care; SDG 9 (Industry, Innovation & Infrastructure) by advancing smart drug-delivery technologies.
- **Potential applications:** Image-guided chemotherapy, combination regimens, and treatment of tumors with high lysosomal activity (e.g., glioblastoma, ovarian).
- **Limitations & next steps:** In-vivo PK/PD, toxicity profiling, scale-up and stability, comparative efficacy vs. standard 5-FU regimens, and regulatory pathfinding.

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