

## Supporting Information

### Repurposing Methylene Blue as Activatable Prodrugs for Precise Disease Theranostics

Junliang Zhou,<sup>\*a,b</sup> Liang Su,<sup>b,c</sup> Tian-Bing Ren,<sup>b</sup> Lin Yuan<sup>\*b</sup>

<sup>a</sup> School of Pharmacy, Hunan University of Chinese Medicine, Changsha, 410208, P. R. China. Email: zjl1046@hnu.edu.cn;

<sup>b</sup> State Key Laboratory of Chemo and Biosensing, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, P. R. China. Email: lyuan@hnu.edu.cn;

<sup>c</sup> Qianjin Pharmaceutical CO., LTD., Zhuzhou, 412400, P. R. China.

**Table S1. Activation strategies for MB-based activatable prodrugs in theranostics**

Activation Strategy	Triggers	Advantages	Limitations	Current Challenges
ROS	H <sub>2</sub> O <sub>2</sub> , HOCl, •OH, ONOO <sup>-</sup>	High pathological relevance; Broadly applicable across cancers and inflammatory diseases; Relatively simple chemical design	Intrinsically low specificity due to ROS presence in multiple pathological processes; Prone to off-target activation and background signal	Developing ROS subtype-selective prodrugs; Improving activation thresholds; Integrating multi-stimuli logic gating to enhance specificity
GSH	Intracellular GSH	Exploits redox imbalance in tumors; Can deplete GSH to amplify oxidative stress and enhance PDT/SDT efficacy	Significant heterogeneity of GSH levels across tumor types and stages; Possible premature activation in normal tissues with high GSH	Achieving tumor-selective activation; Balancing stability vs responsiveness; Integrating dual-trigger systems
Enzyme	TYR, FAP $\alpha$ , NQO1, HDAC	High molecular specificity; Suitable for personalized theranostics based on biomarker expression	Tumor heterogeneity leads to uneven enzyme expression; Enzyme activity may vary temporally and spatially	Identifying robust and universal enzymatic biomarkers; Overcoming heterogeneous activation
Hypoxia	Low O <sub>2</sub> , reductive environment	Highly relevant to solid tumors; Can synergize with PDT-induced hypoxia; Enables selective activation in poorly perfused regions	Hypoxia heterogeneity within tumors; Relatively slow activation kinetics	Combining with oxygen modulation strategies; Integrating feedback amplification mechanisms
NIR light	650–900 nm light	Precise spatiotemporal control; Noninvasive; Enables real-time imaging-guided therapy	Limited tissue penetration (especially in NIR-I window); Potential phototoxicity; Dependence on external equipment	Improving light delivery systems; Minimizing photodamage while maintaining efficacy
Ultrasound	Ultrasound	Deep tissue penetration; Noninvasive; Clinically translatable; Suitable for deep-seated tumors	Limited intrinsic targeting specificity; Activation efficiency may be moderate; ROS generation can be oxygen-dependent	Enhancing tumor accumulation via delivery systems; Improving activation efficiency
Bioorthogonal	Exogenous	Exceptional selectivity; Minimal interference with biological systems; Precise spatiotemporal control	Requires delivery of multiple components; Complex pharmacokinetics; Potential mismatch in reaction partners in vivo	Improving in vivo reaction kinetics; Simplifying system design; Ensuring co-localization of reactants

**Table S2. MB-based activatable prodrugs for disease theranostics**

Prodrugs No.	Triggering mechanism	Therapeutic payload	Disease model	Key outcome
1	HClO; HRP+H <sub>2</sub> O <sub>2</sub> ; Ultrasound	Melphalan	Melanoma	Significant tumor inhibition
2	ROS	Camptothecin (CPT)	Metastatic cervical cancer	99.9% tumor inhibition; 100% survival; No systemic toxicity
3	ROS	Doxorubicin (DOX)	Liver cancer (orthotopic)	Enhanced tumor retention; Extended survival time
4	hROS	Chlorambucil (CLB)	HepG2 tumors	Significant tumor growth inhibition
5	ROS	MB (NIRF imaging agent)	HeLa tumor	High tumor-to-background ratio; Enables fluorescence-guided surgery
6	H <sub>2</sub> O <sub>2</sub>	MB (photosensitizer)	HepG2 tumors	Enhanced ROS generation; Improved PDT efficacy; Good biosafety
7	HClO	Indomethacin; MB (photosensitizer)	Lung cancer, metastasis	Induces ICD; Enhances CD8 <sup>+</sup> T cell infiltration
8	HOCl; ONOO <sup>-</sup>	4-Hydroxybenzaldehyde (4-HBA); MB (photosensitizer)	Melanoma	98.8% tumor inhibition; No recurrence after single treatment
9	HClO	QDAU5; MB (photosensitizer)	Melanoma	Induces apoptosis and immune activation
10	GSH	MB (photosensitizer)	HeLa tumors	Effective PDT; No systemic toxicity
11	GSH	Cisplatin; MB (photosensitizer)	Drug-resistant A549 tumors	96.4% tumor inhibition; Reverses drug resistance
12	GSH	R837 (immunoadjuvant); MB (sonosensitizer)	CT26 tumors (bilateral model)	Activates immune response; Inhibits primary & distant tumors
13	GSH	CPT; MB (photosensitizer)	4T1 breast cancer	Nearly complete tumor inhibition; Synergistic PDT + chemotherapy
14	Hypoxic	AZO-PRO (PROTAC); MB (photosensitizer)	MCF-7 tumors	Enhanced antitumor efficacy; No systemic toxicity
15	TYR	MB (photosensitizer)	Melanoma	Selective photokilling
16	FAP $\alpha$	MB (photosensitizer)	4T1 tumors	GSH depletion; Effective PDT
17	FAP $\alpha$	MB (photosensitizer)	4T1 tumors (bilateral model)	Activates immune response; Inhibits primary & distant tumors
18	NQO1	MB (NIRF imaging agent)	A549 tumors	Selective tumor theranostics
19	HDAC6	MB (photosensitizer; MAO-A inhibitor)	Glioma	Induces apoptosis; Inhibits migration and invasion
20	NIR light	STAT3 inhibitor; MB (photosensitizer)	MDA-MB-231 tumors	92.7% tumor suppression; Inhibits metastasis
21	NIR light	TMG (alkalizing agent); MB (photosensitizer)	4T1 tumors	Induces mitochondrial dysfunction and apoptosis
22	NIR light	MB (photosensitizer)	4T1 tumors	Effective tumor inhibition
23	NIR light	Nitrogen mustard; MB (photosensitizer)	Melanoma	Strong cytotoxicity; Effective tumor inhibition
24	Ultrasound	IDO-1 inhibitor; MB (sonosensitizer)	4T1 TNBC tumors	Induces ICD; Suppresses recurrence and metastasis
25	Ultrasound	Ferrocene; MB (sonosensitizer)	4T1 tumors	Induces ferroptosis; Complete tumor inhibition

26	Ultrasound	BRD4 degrader; MB (sonosensitizer)	4T1 tumors	Efficient BRD4 degradation; Enhanced tumor suppression
27	Bioorthogonal trigger	MB (sonosensitizer)	EMT-6 tumors	Enhanced tumor suppression
28	Bioorthogonal trigger	MB (sonosensitizer)	HeLa cells	Strong cytotoxicity
29	ROS	Iron chelator	Skin allograft	Effectively suppressed immune rejection; Reduced skin inflammation
30	ROS	5-ASA	IBD	Noninvasive diagnosis; Reduced inflammation
31	HOCl	Ibuprofen	Osteoarthritis	Reduced inflammation; Cartilage protection
32	HOCl	Glycine	HIRI	Reduced TNF- $\alpha$ ; Alleviated liver injury
33	HOCl	Neurotransmitters (dopamine, 5- hydroxytryptamine); Antidepressants (fluoxetine)	Depression model	Restored BDNF; Reduced neuroinflammation
34	HOCl	Riluzole	Ischemic stroke	Reduced ROS/LPO; Neuroprotection
35	F <sup>-</sup>	MB (photosensitizer)	Dental plaque	Enhanced antibacterial PDT
36	HOCl	MB (photosensitizer); CTAB	Bacterial infection	Dual antibacterial mechanism; Effective against <i>S. aureus</i> & <i>E. coli</i>