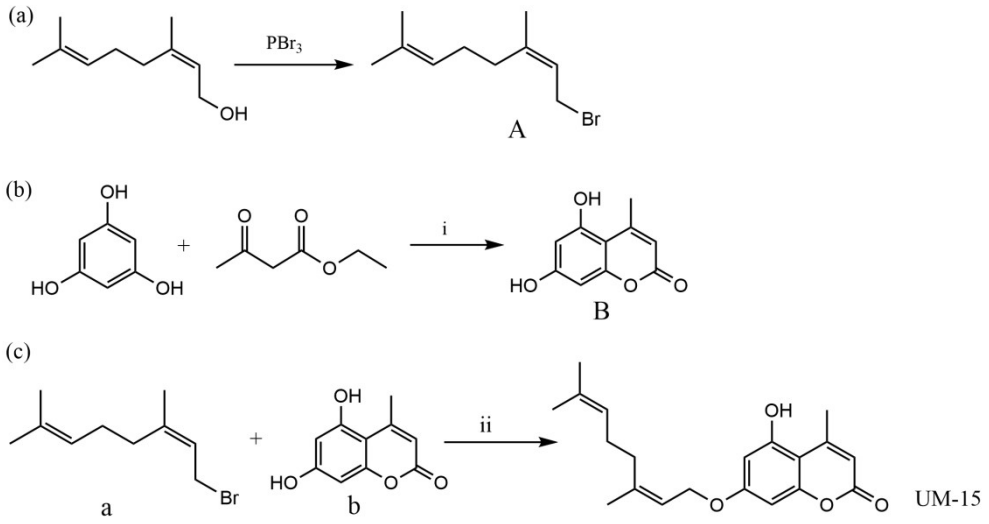
Supporting information

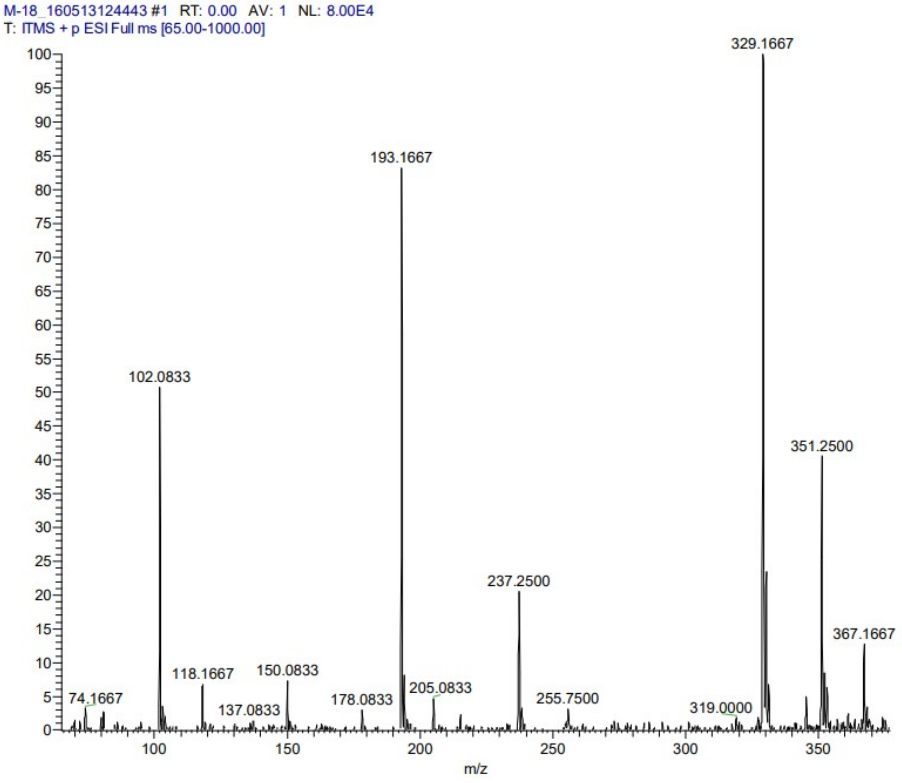
Intravesically Cascade Delivery of Active Monoterpene Coumarin for Bladder Cancer Therapy

Synthesis of UM-15



Scheme S1. Synthetic route of UM-15. (i) $POCl_3$, stirring for 1h at 40℃; (ii) K_2CO_3, KI, anhydrous acetone, rt for 2.5 h.

UM-15 was synthesized following previous literatures with slight modification (Scheme S1).[1, 2] Firstly, nerol (1.72 g，20.0 mmol) and catalytic dose of pyridine were mixed in anhydrous ether (50 mL), then PBr_3 (2.71g，10.0 mmol) was added in slow drops with nitrogen protection at 0℃. After 0.5 h reaction, the reaction was returned to room temperature and continued for another hour. The crude product was extracted with ethyl acetate to obtain substrate A as brown oil (Yield 89.3%), which was applied for UM-15 synthesis directly. Next, to prepare another substrate B, phloroglucinol (1.0 eq.) and catalytic dose of $POCl_3$ were stirred with ethyl diacetoacetate (1.1 eq.) in anhydrous acetone for 1.0 h at 40℃, and then further purified by chromatography on a silica gel column eluted with $CHCl_3$–MeOH (6:1, v/v) to obtain substrate B as white power (Yield 88.1%). ^{1}H-NMR (600 MHz，DMSO) δ:2.48 (s, 3H), 5.83 (s, 1H), 6.16(s, 1H), 6.25 (s, 1H), 10.29 (s, -OH), 10.51 (s, -OH). Finally, compound B (1.0 eq.), compound A (1.2 eq.), catalytic dose of KI, and K_2CO_3 (2.0 eq.) were added in anhydrous acetone, and stirred 2.5 h at room temperature. Then the solution was extracted with ethyl acetate, and subsequently eluted with $CHCl_3$–MeOH (5:1, v/v) on a silica gel column to prepare UM-15 (Yield 37.5%) as white powder. (+)HRESIMS $[M+H]^+$ m/z 329.1667; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.83 (s, 1H), 6.76 (s, 1H), 6.33 (s, 1H), 5.95(s, 1H), 5.49 (t, 1H), 4.53 (d, 2H), 2.56 (s, 3H), 2.13 (m, 4H), 1.8 (s, 3H) , 1.66 (s, 3H) , 1.59 (s, 3H). ^{13}C-NMR (150 MHz，$CDCl_3$) δ：17.84, 23.62, 24.71, 25.85, 26.59, 32.53, 65.83, 96.47, 96.94, 104.84, 110.54, 119.37, 123.52, 132.61, 142.27, 156.25, 156.71, 159.03, 160.50, 162.50.


M-18_160513124443 #1 RT: 0.00 AV: 1 NL: 8.00E4
T: ITMS + p ESI Full ms [65.00-1000.00]
329.1667
193.1667
102.0833
351.2500
237.2500
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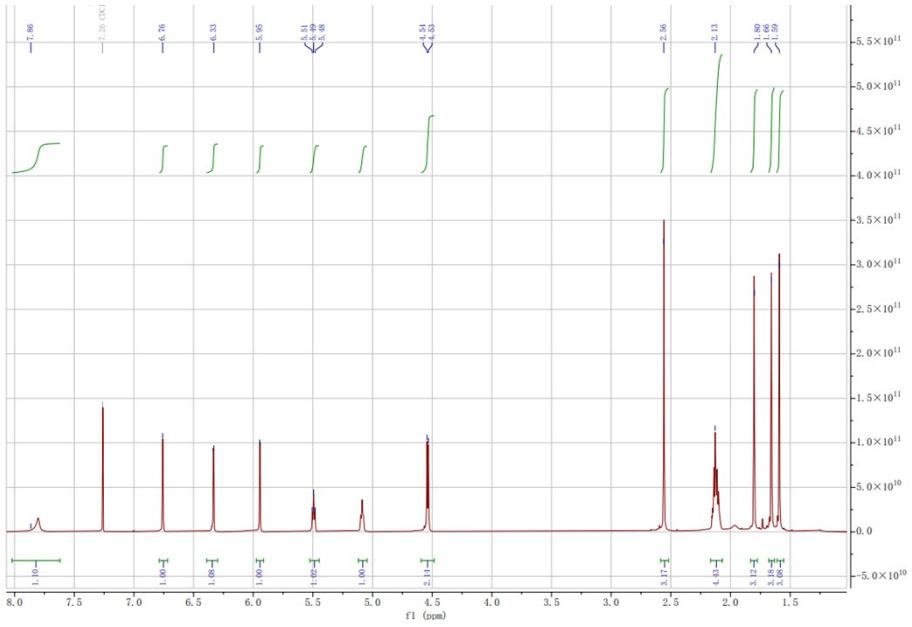
150.0833
205.0833
74.1667
137.0833
178.0833
255.7500
319.0000
m/z


f1 (ppm)

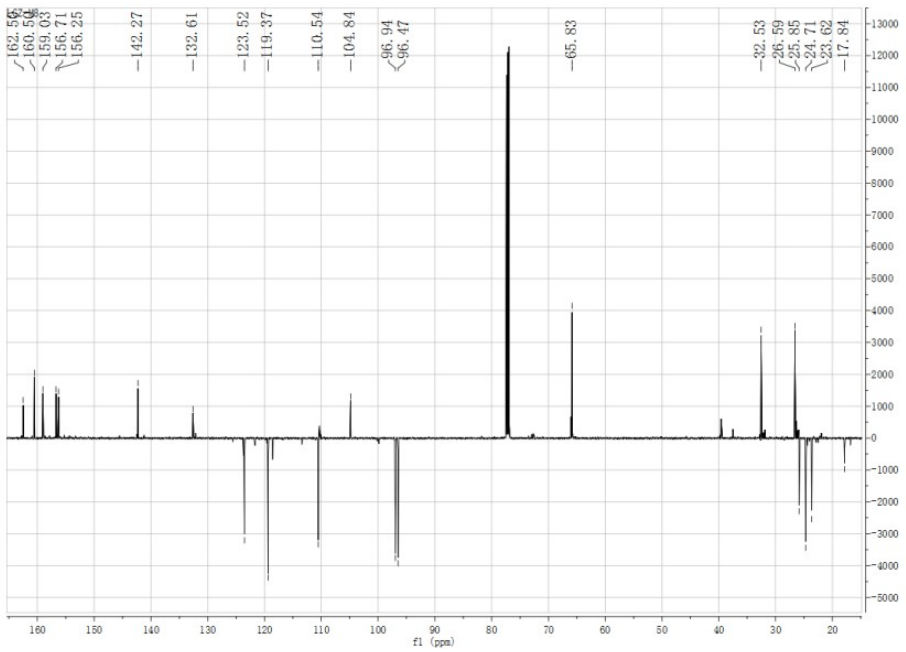


Figure S1. ESI-MS, ^{1}H NMR and ^{13}C NMR spectrum of UM-15

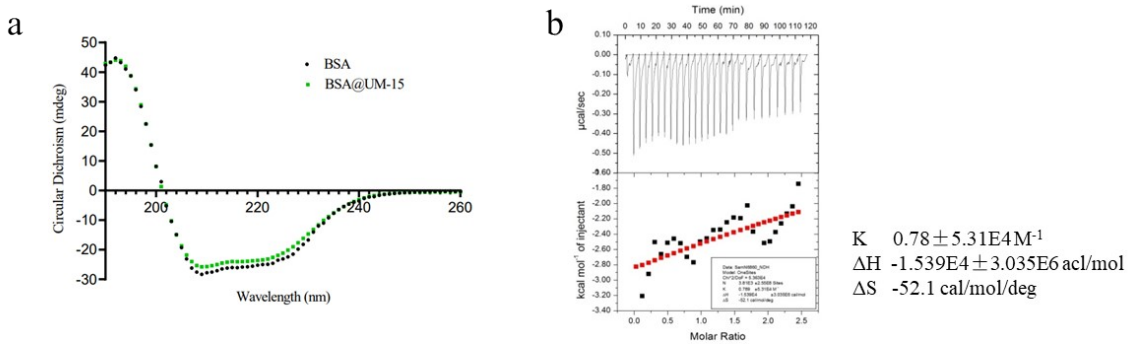


Figure S2. a. CD spectra of the BSA@UM-15 complex; b. Isothermal titration trace heat curve of BSA titrated by UM-15 at 298 K .

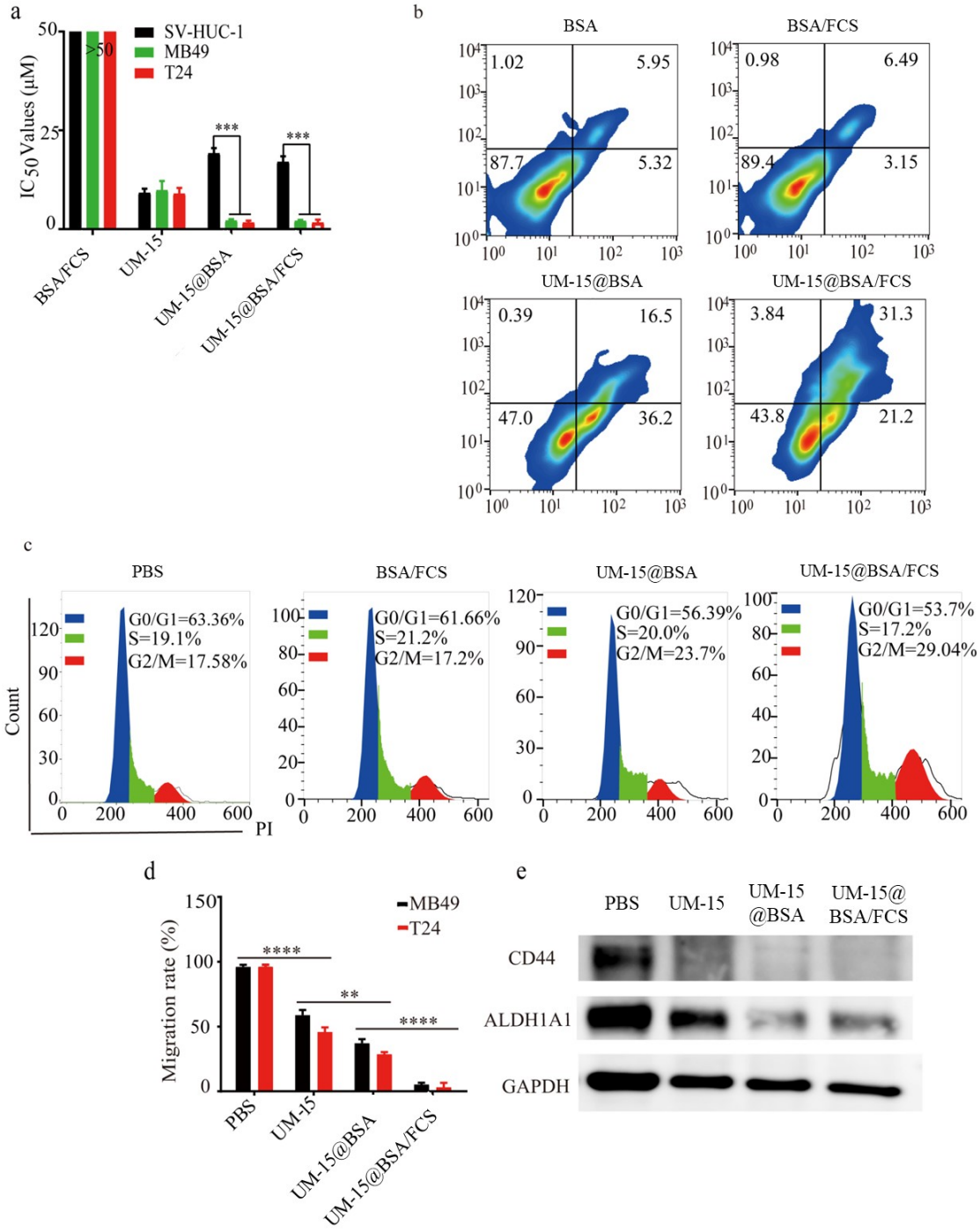


Figure S3. The therapeutic mechanisms of UM-15-loading agents. (a) IC_{50} values of BSA /FCS, free UM-15, UM-15@BSA and UM-15@BSA /FCS against different cell lines (T24, MB49, and SV-HUC-1). (b-c) The evaluation of apoptosis and cell cycle arrest by different $BSA_{S\text{-}S}$-based nanostructures using annexin V-FITC/PI co-staining and flow cytometry analysis. (d) The statistical data of wound-healing assay for the evaluation of migration. (e) The expression levels of tumor stemness-associated proteins were detected by Western blot.

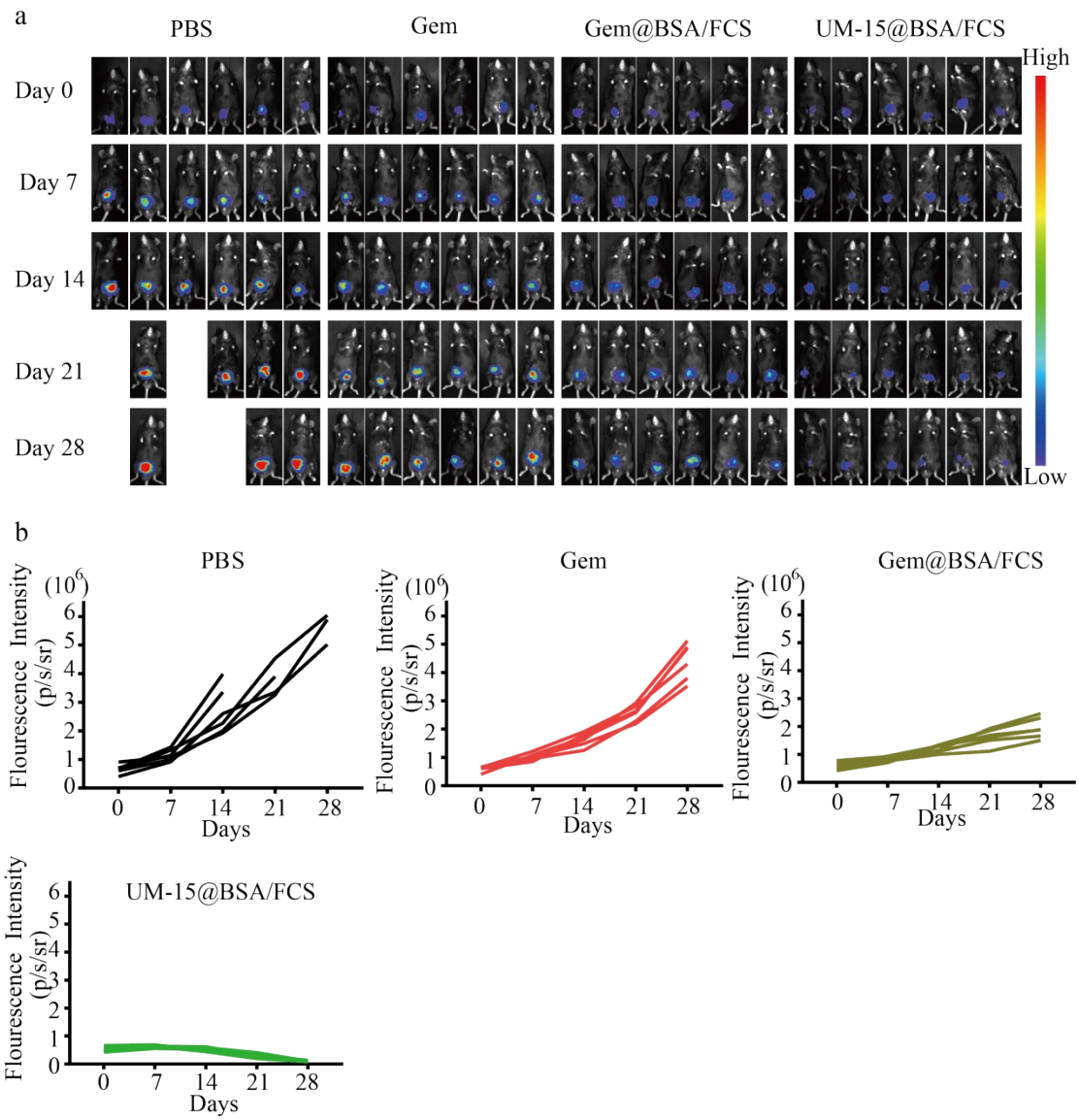


Figure S4. Intravesical instillation of Gem, Gem@BSA /FCS or UM-15@BSA/FCS to treat orthotopic BCa tumors. (a) In vivo bioluminescence images to track the growth of tumor cells after various treatments. (b) The bioluminescence signal intensities of tumors during intravesical treatment.

References

[1] Hu, Y., Zhong, Y., Li, J., Cai, L., Li, H. J., Topics, R., *Review of Progress in Coloration and Related Topics* **2011,** *127*, 1478.

[2] Firdaus, M., Marliyana, S. D., Razak, M. F., THE 14TH JOINT CONFERENCE ON CHEMISTRY 2019, 2020.