**Characterizations of chemotherapeutic dihydromyricetin with enhanced anti-tumor activity and biosafety for muscle invasive bladder cancer**

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**Figure S1.** Dihydromyricetin (DHM) motivated macrophage polarization in vitro. (a, c) Representative flow cytometry images of CD80 and CD86 staining after DHM chemotherapy; (b, d) Quantitative results of CD80 and CD86 positively stained cells. \*\*\**P* < 0.001.

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**Figure S2.** Dihydromyricetin (DHM) inhibited proliferation and promoted apoptosis of BCa in vivo xenografts. (a) Representative Ki67 staining images of the tumor samples. Scale Bar: 50 μm. (b) Representative TUNEL staining images of the tumor samples. Scale Bar: 50 μm.

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**Figure S3.** Dihydromyricetin (DHM) possessed remarkable in vivo hemocompatibility. (a) Quantitative results of enzymatic activity of ALT, AST and GGT. (b) Representative results of the concentration of CRE and TBIL. N.S. represented *P* > 0.05.



**Figure S4.** (a) The West-blot quantitative analysis results of CDK2, CDK4, Cyclin D1 in T24. (b) The West-blot quantitative analysis results of CDK2, CDK4, Cyclin D1 in

UMUC3. (c) The West-blot quantitative analysis results of Ki67. (d) The West-blot quantitative analysis results of Caspase 3, Caspase 6, Caspase 9 in T24. (e) The West-blot quantitative analysis results of Caspase 3, Caspase 6, Caspase 9 in UMUC3. (f) The immunofluorescence staining quantitative analysis results of Ki67. (g, h, j) The West-blot quantitative analysis results of E-cadherin, N-cadherin, Vimentin, Snail. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

**Table S1.** Primer sequences used for qRT-PCR

|  |  |  |
| --- | --- | --- |
| Primer name | sequences 5’-3’ | Tm |
| GAPDH-F | TGTGGGCATCAATGGATTTGG | 60.9 ºC |
| GAPDH-R | ACACCATGTATTCCGGGTCAAT | 61.4 ºC |
| CDK2-F | TGTTTAACGACTTTGGACCGC | 61.1 ºC |
| CDK2-R | CCATCTCCTCTATGACTGACAGC | 61.5 ºC |
| CDK4-F | GGGGACCTAGAGCAACTTACT | 60.3 ºC |
| CDK4-R | CAGCGCAGTCCTTCCAAAT | 60.4 ºC |
| Cyclin E1-F | TCGCATCAAACTCTCTGGCTA | 60.9 ºC |
| Cyclin E1-R | TGAGCGACTAAACTCACCACT | 60.8 ºC |
| Cyclin D1-F | GCTGCGAAGTGGAAACCATC | 61.6 ºC |
| Cyclin D1-R | CCTCCTTCTGCACACATTTGAA | 60.8 ºC |
| P53-F | AACTGCGGGACGAGACAGA | 62.9 ºC |
| P53-R | AGCTTCAAGAGCGACAAGTTTT | 60.7 ºC |
| Vimentin-F | GCCCTAGACGAACTGGGTC | 61.4 ºC |
| Vimentin-R | GGCTGCAACTGCCTAATGAG | 61.1 ºC |
| E-cadherin-F | CGAGAGCTACACGTTCACGG | 62.6 ºC |
| E-cadherin-R | GGGTGTCGAGGGAAAAATAGG | 60.1 ºC |
| N-cadherin-F | TTTGATGGAGGTCTCCTAACACC | 61.3 ºC |
| N-cadherin-R | ACGTTTAACACGTTGGAAATGTG | 60.2 ºC |
| Snail-F | TGTGACAAGGAATATGTGAGCC | 60.0 ºC |
| Snail-R | TGAGCCCTCAGATTTGACCT | 61.5 ºC |
| Caspase 3-F | AGAGGGGATCGTTGTAGAAGTC | 60.6℃ |
| Caspase 3-R | ACAGTCCAGTTCTGTACCACG | 61.4℃ |
| Caspase 6-F | GAGCACGTTGGATATGATGGTG | 61.1℃ |
| Caspase 6-R | GGTCCACTTACATCCTCGATCTA | 60.4℃ |
| Caspase 9-F | ATGTCGGACTACGAGAACGAT | 60.4℃ |
| Caspase 9-R | TGATGCGTGAGGGGTCGAT | 63.0℃ |

**Table S2.** List of primary antibodies and secondary antibodies

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| --- | --- | --- | --- |
| name | species | dilution | resources |
| GAPDH | Mouse | 1:1000 | Abcam, USA, Cat. # ab181602 |
| CDK2 | Rabbit | 1:1000 | Abcam, USA, Cat. # ab32147 |
| CDK4 | Rabbit | 1:1000 | Abcam, USA, Cat. #ab108357 |
| Cyclin D1 | Rabbit | 1:1000 | Abcam, USA, Cat. #ab16663 |
| Ki67 | Rabbit | 1:100 | Abcam, USA, Cat. # ab15580 |
| E-cadherin | Rabbit | 1:1000 | Abcam, USA, Cat. #ab194982 |
| N-cadherin | Rabbit | 1:1000 | Abcam, USA, Cat. #ab18203 |
| Snail | Rabbit | 1:1000 | Abcam, USA, Cat. #ab229701 |
| Vimentin | Rabbit | 1:1000 | Abcam, USA, Cat. #ab93547 |
| Caspase 3 | Rabbit | 1:1000 | Abcam, USA, Cat. #ab51772 |
| Caspase 6 | Rabbit | 1:1000 | Abcam, USA, Cat. #ab32053 |
| Caspase 9 | Rabbit | 1:1000 | Abcam, USA, Cat. #ab219590 |
| Anti-Mouse-IgG (H+L)-HRP | Goat | 1:10000 | Sungene Biotech, China, Cat. #LK2003 |
| Anti-Rabbit-IgG(H+L)-HRP | Goat | 1:10000 | Sungene Biotech, China, Cat. #LK2001 |

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