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SHORT REPORT

Discovery and anticancer evaluation of a formononetin derivative against gastric cancer SGC7901 cells

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Summary

Background Gastric cancer (GC) is the second most common cause of cancer-related death worldwide. Novel anticancer drugs against gastric cancer are urgently needed. *Methods* Compound **10** was designed and synthesized via a molecular hybridization strategy based on the natural product formononetin. It was evaluated for their antiproliferative activity against three gastric cancer cell lines (SGC7901, MKN45 and MGC803). *Results* Derivative **10** displayed potently antiproliferative activity with an IC_{50} value of 1.07 μM against SGC7901 cells. Derivative **10** could inhibit the growth and migration against gastric cancer SGC7901 cells through the Wnt/β-Catenin and AKT/mTOR pathways. From the in vivo expremints, it could effectively inhibited SGC7901 xenograft tumor growth in vivo without significant loss of the body weight. *Conclusion* Derivative **10** is an novel antitumor agent with potential for further clinical applications to treat gastric cancer.

Keywords Gastric cancer · Formononetin · SGC7901 · Growth · Migration

Introduction

Gastric cancer (GC) as the second leading cause of cancer-related death worldwide is relatively high in eastern Asia [1]. In China, as the dominant types of cancer in the age group 60 to 74 years, gastric cancer is the leading cause of death and a major digestive system public health problem, with 0.50 million deaths and 0.68 million new cases in 2015 [2]. Although targeted chemotherapy has increased long-term survival of patients with gastric cancer, the clinical application has been limited owing to the severe adverse effects [3]. Therefore, novel drugs with high anticancer efficiency against gastric cancer are urgently needed.

Formononetin isolated from the red clover displayed a lot of pharmacological effects such as vasorelaxant, antioxidant, anticancer, antiinflammatory and neuroprotective activies by regulating the mitogen-activated protein kinase signal pathway or estrogen receptor [4–6]. Formononetin could induce cell cycle arrest by decreasing cyclin D1 mRNA and protein expression in human breast cancer in vitro and in vivo [7]. Formononetin in combination with temozolomide displayed a synergistic role on Glioma C6 cells through inducing tumor cells apoptosis [8]. However, formononetin as a natural product displayed weak or no activity (Fig. 1) with IC_{50} values >30 μM against MGC803 cells, PC3 cells and MCF7 cells [9–11]. In order to discovery more potent antitumor compounds based on the natural formononetin, the structural modification of formononetin was very necessary.

Coumarins exhibited a wide range of pharmacological activities in drug discovery [12–16]. The promising biological profile and easy synthetic modification have stimulated the design and development of coumarin-based derivatives as potential antitumor agents [17]. Molecular hybridization is a useful strategy in drug design and development based on the combination of pharmacophoric moieties of different bioactive units to produce a new hybrid with improved affinity and efficacy, when compared to the parent drugs [18, 19]. From these interesting findings, we introduced the coumarin unit to design a potent anticancer molecule based on the natural formononetin via a molecular hybridization strategy (Fig. 2).

Jian-Ning Yao and Xue-Xiu Zhang contributed equally to this work.

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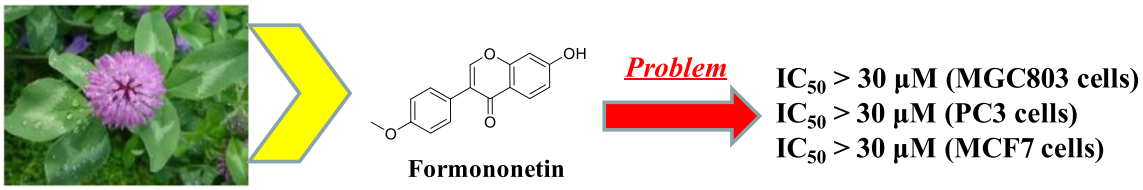


Fig. 1 The main problem of formononetin as a natural and anticancer product

In this work, the synthesis and anticancer mechanisms of the formononetin-coumarin hybrid were investigated.

Materials and methods

^{1}H and ^{13}C NMR spectra of compound 8

White solid, m.p.: 104~106 °c, yield:50.66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99 (d, J = 9.5 Hz, 1H), 7.63 (d, J = 8.6 Hz, 1H), 7.10–6.82 (m, 2H), 6.29 (d, J = 9.5 Hz, 1H), 4.19 (t, J = 6.0 Hz, 2H), 3.67 (t, J = 6.6 Hz, 2H), 2.28 (p, J = 6.3 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.95, 160.74, 155.82, 144.76, 130.01, 113.42–112.82 (m), 101.73, 66.56, 40.60, 40.39, 40.18, 39.97, 39.77, 39.56, 39.35, 32.06, 31.47. Found, *m/z*: 282.9978 $[M + H]^+$. $C_{12}H_{12}BrO_3$. Calculated: 282.9970.

^{1}H and ^{13}C NMR spectra of compound 9

White solid, m.p.: 100~103 °c, yield:41.34%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, J = 9.5 Hz, 1H), 7.31 (d, J = 8.3 Hz, 1H), 6.86–6.67 (m, 2H), 6.19 (d, J = 9.5 Hz, 1H), 4.21–4.04 (m, 2H), 3.51 (dt, J = 27.8, 6.4 Hz, 2H), 2.29 (p, J = 6.1 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.84, 160.11, 154.85, 142.32, 127.80, 112.29, 111.73, 100.55, 76.32, 76.00, 75.68, 64.86, 30.95, 28.50. Found, *m/z*: 246.0873 $[M + H]^+$. $C_{12}H_{12}N_3O_3$. Calculated: 246.0879.

^{1}H and ^{13}C NMR spectra of compound 10

White solid, m.p.: 156~158 °c, yield:32.89%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (s, 1H), 8.36 (s, 1H), 8.01 (dd, J = 21.3, 9.2 Hz, 2H), 7.62 (d, J = 8.6 Hz, 1H), 7.53 (d, J = 8.6 Hz, 2H), 7.35 (d, J = 2.1 Hz, 1H), 7.13 (dd, J = 8.9, 2.1 Hz, 1H), 6.99 (dd, J = 13.6, 5.4 Hz, 3H), 6.91 (dd, J = 8.6, 2.2 Hz, 1H), 6.29 (d, J = 9.5 Hz, 1H), 5.33 (s, 2H), 4.59 (t, J = 6.8 Hz, 2H), 4.11 (t, J = 5.9 Hz, 2H), 3.80 (s, 3H), 2.44–2.23 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.09, 162.83, 161.92, 160.74, 159.49, 157.79, 155.81, 153.99, 144.77, 142.33, 130.55, 129.97, 127.47, 125.59, 124.51, 123.87, 118.27, 115.67, 114.10, 113.12, 113.06, 112.95, 101.72, 65.91, 62.37, 55.63, 47.14, 29.67. Found, *m/z*: 552.1779 $[M + H]^+$. $C_{31}H_{26}N_3O_7$. Calculated: 552.1771.

MTT assay

Three gastric cancer cell lines (SGC7901, MKN45 and MGC803) were bought from the first affiliated hospital of zhengzhou university and shanghai institute of biochemistry and cell biology. 1000 Cells were seeded in 96-well plates per well. For each well, 20 μl 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide

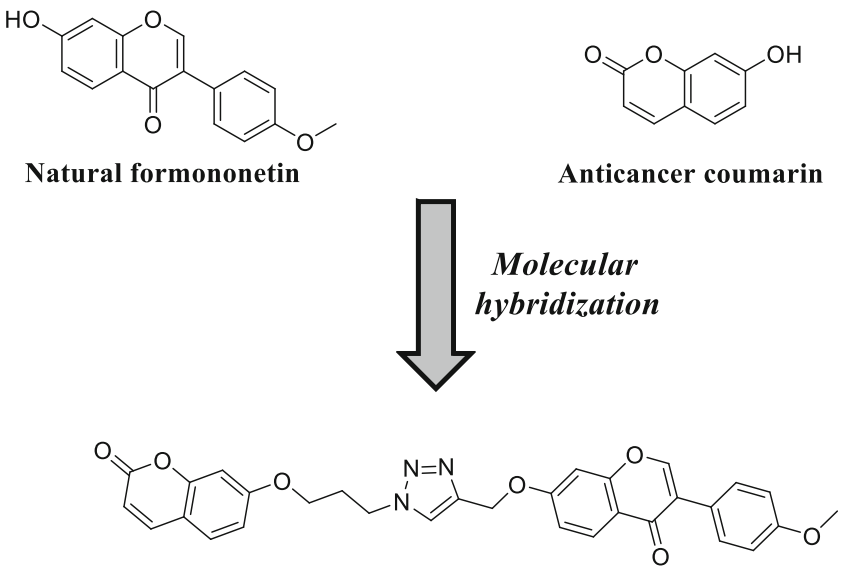
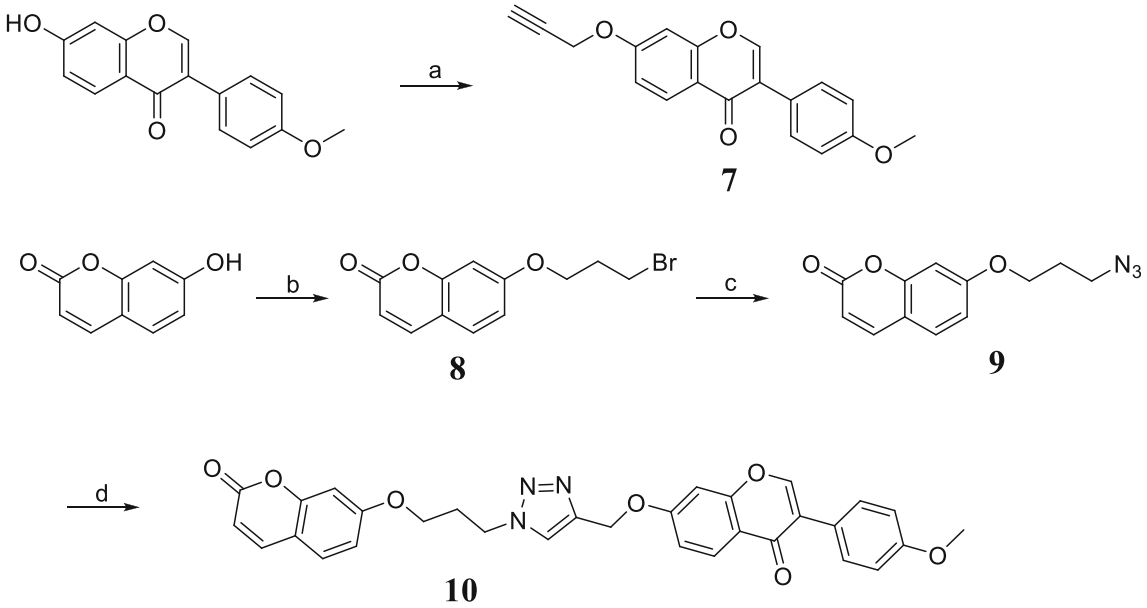


Fig. 2 Molecular hybridization strategy to design the targeted compound



Scheme 1 Synthesis of the formononetin-coumarin hybrid **10**. Reagents and conditions: **a** propargyl bromide, NaOH, acetone, reflux; **b** 1,3-dibromopropane, K_2CO_3, CH_2Cl_2, reflux; **c** NaN_3, CH_3CN, reflux; **d** intermediate **7**, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, DMSO:H_2O (1:1), r.t

(MTT, 5 mg/ml) was added and plates were incubated at 37 °C for 1 h. After removing the supernatant, 100 μl DMSO per well was added [20].

Wound healing

SGC7901 cells were seeded in 6-well plates and the formononetin-coumarin hybrid **10** was added for 48 h. Wounds were created using a pipette tip and medium was added for 24 h. Each experiment was repeated at least three times [21].

SIRT 1 inhibition assay in vitro

The peptide substrate is incubated with human recombinant SIRT1 along with its cofactor NAD+. Fluorophore was detected using an excitation wavelength of 360 nm and an emission wavelength of 460 nm. The reactions were suited to high-throughput screening, and the assay was performed in the 96-well microplate by reported references [22, 23].

Western blot analysis

SGC7901 cells were lysed in a RIPA lysis buffer and centrifuged at 15000 rpm for 30 min. The protein samples were electrophoresed on SDS-PAGE and transferred to PVDF membrane. Then, PVDF membrane was incubated with corresponding primary and secondary antibodies. The proteins were visualized using chemiluminescence (ECL) detection reagents [24].

Animals study in vivo

Animals experiments were processed according to protocols and guidelines established by the ethics committee of

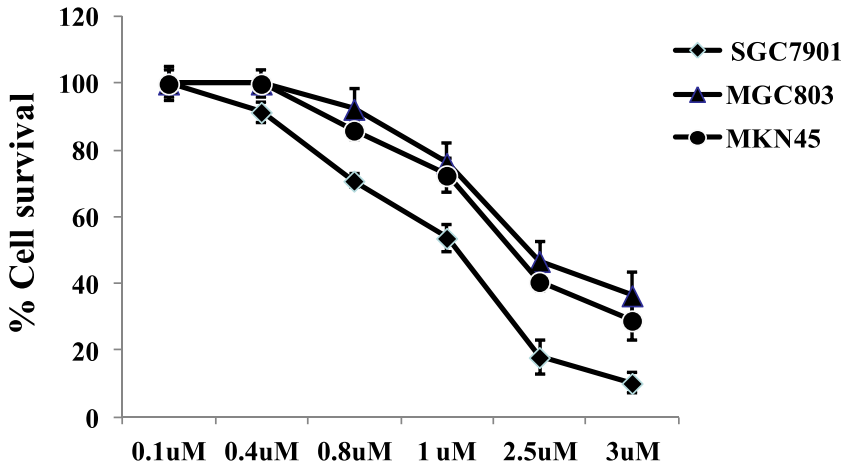


Fig. 3 Gastric cancer cells (SGC7901, MKN45 and MGC803) were treated with the hybrid **10** for 48 h

zhengzhou university. Mice were subcutaneously implanted with SGC7901 cells (1 X 10^7 cells per mouse) on the right flank of nude mice. Once tumor volumes reached to 100 mm^3, the mice were randomly divided into two groups ($n = 5$ mice for each group). The treatment group received intragastric administration of formononetin-coumarin hybrid **10** per day for a period of 21 days [25].

Results

Synthesis of analogue 10

The novel formononetin hybrid **10** was synthesized as illustrated in Scheme 1. Natural formononetin was coupled with propargyl bromide in acetone in the presence of sodium hydroxide to obtain formononetin intermediate **7**. In addition, coumarin intermediate **9** was obtained via the nucleophilic substitution reaction of commercially available coumarin with 1,3-dibromopropane. The target formononetin-coumarin hybrid **10** was synthesized from the alkyne intermediate **7** and azide intermediate **9** using a click reaction in the presence of copper(II) sulfate pentahydrate.

Analogue 10 inhibited gastric cancer cells growth

Gastric cancer (GC) has been the fourth most common cancer and the second leading cause of cancer-related death in the world [26]. It is very necessary to discovery anticancer drugs to treat gastric cancer. In this work, three gastric cancer cell lines (SGC7901, MKN45 and MGC803) were selected to test the cell viability and the formononetin-coumarin hybrid **10** (0.1 μM, 0.4 μM, 0.8 μM, 1 μM, 2.5 μM and 3 μM) was added for 48 h. As shown in Fig. 3, the formononetin-coumarin hybrid **10** could inhibit gastric cancer cells growth in a concentration-dependent manner. Especially, the formononetin-coumarin hybrid **10** displayed potently antiproliferative activity with an IC_{50} value of 1.07 μM against SGC7901 cells. However, formononetin displayed the weak activity against three cell lines (SGC7901, MKN45 and MGC803) with IC_{50} values >20 μM. This result suggests that coumarin moiety may play a synergistic role for inhibitory activity against cancer cells.

Analogue 10 inhibited SIRT1 expression against SGC7901 cells

Sirtuin 1 (SIRT1) as a member of SIRT family is up-regulated in several types of tumors [27]. As a nicotinamide adenine dinucleotide-(NAD^+-) dependent histone deacetylase, SIRT1 functions as a master regulator of ageing, apoptosis, and stress response [28]. Recently, SIRT1 has been an important target to discovery SIRT1 inhibitors and anticancer agents [29].

To explore the SIRT1 enzyme inhibitory activity of hybrid **10**, the reported SIRT1 inhibition assay in vitro was used [30]. As shown in Fig. 4, formononetin-coumarin hybrid **10** displayed a high degree of inhibitory activity toward SIRT1 with an IC_{50} of 2.52 μM. The tumor suppressor p53 was identified as the critical target of the SIRT1 inhibitor–induced cell death in cancer cells [31]. From the western blot results, the expression of

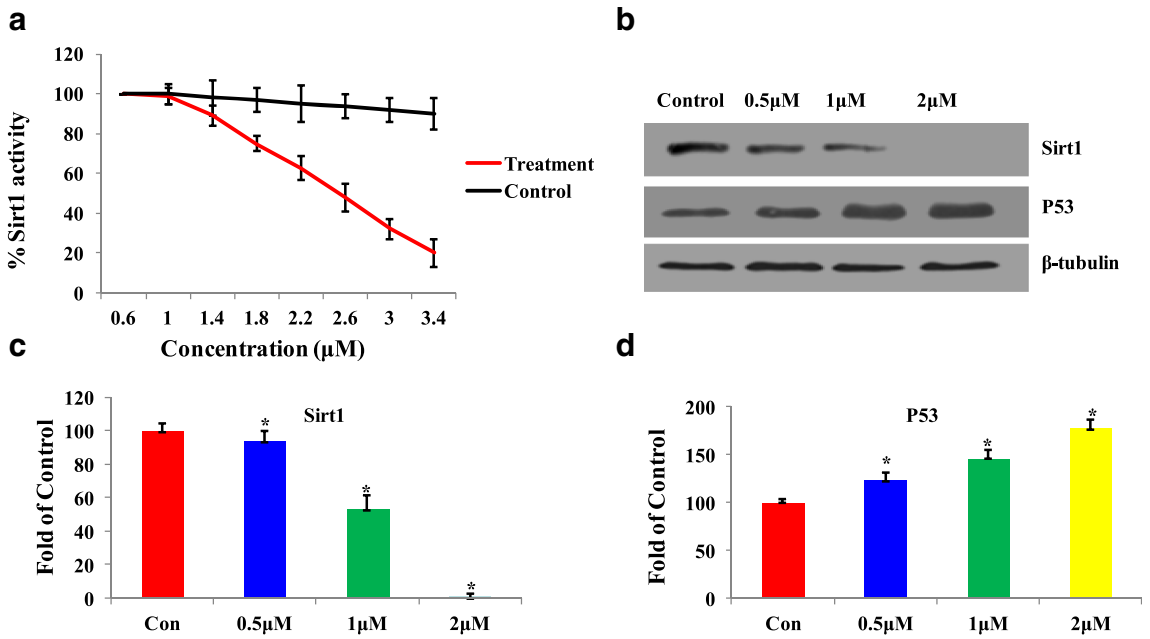


Fig. 4 **a** In vitro SIRT1 inhibition assay; **b** The expression of SIRT1 and p53 treated with hybrid **10** (control, 0.5 μM, 1 μM, and 2 μM) in SGC7901 cells; **c** Fold of control for SIRT1 protein; **d**: Fold of control for P53 protein. * $P < 0.05$ was considered significant

SIRT1 protein was decreased and total p53 expression level was upregulated after treated with formononetin-coumarin hybrid **10**. All these expreiments illustrated that formononetin-coumarin hybrid **10** might be a novel SIRT1 inhibitor.

Analogue 10 inhibited SGC7901 cells migration

SGC7901 cell migration was evaluated by wound healing and western blot experiments. Microphotographs showed that untreated gastric cancer SGC7901 cells filled most of the wounded area for 48 h after scratching the cell monolayer, whereas treatment with hybrid **10** (1 μM**)** markedly suppressed repairment of the wound (Fig. 5). In addition, we also examined the protein expression of migration-related makers such as E-cadherenin and N-cadherenin [32]. Based on the results from Fig. 5, the expression of E-cadherin was upregulated and the expression of N-cadherence was downregulated by formononetin-coumarin hybrid **10**. All these result suggests that hybrid **10** could inhibit the migration process in SGC7901 tumor cells.

Analogue 10 regulated the Wnt/β-catenin pathway in SGC7901 cells

Wnt/β-Catenin pathway displayed an important role in various cellular process including cell proliferation and migration [33]. In Wnt/β-Catenin signaling pathway, intracellular levels of β-catenin are regulated by a multiprotein complex encompassing kinases such as glycogen synthase kinase-3β (GSK-3β). In the nucleus, β-catenin binds to members of the TCF family of transcription factors, such as TCF-4 protein [34].

Based on the migration results and western bolt analysis above, we also explore whether formononetin-coumarin hybrid **10** could regulate Wnt/β-Catenin pathway in SGC7901 cells. Western blot assay was performed to study the expression levels of β-catenin, Wnt5α, phospho β-catenin, GSK-3β,

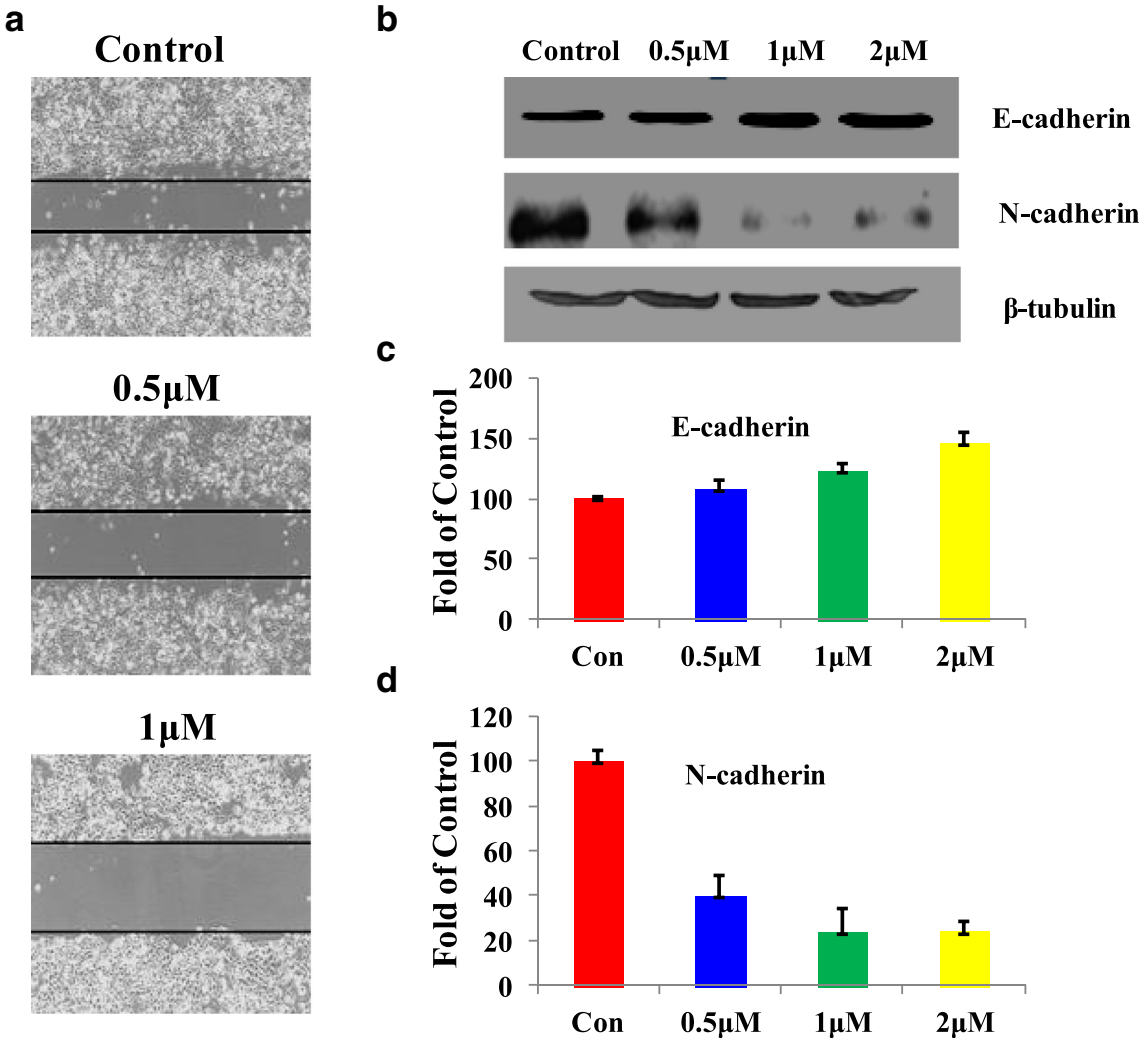


Fig. 5 **a** Effect of hybrid **10** on SGC7901 cells migration; **b** The expression of migration related markers in SGC7901 cells; **c** Fold of control for E-cadherin protein; **d** Fold of control for N-cadherin protein

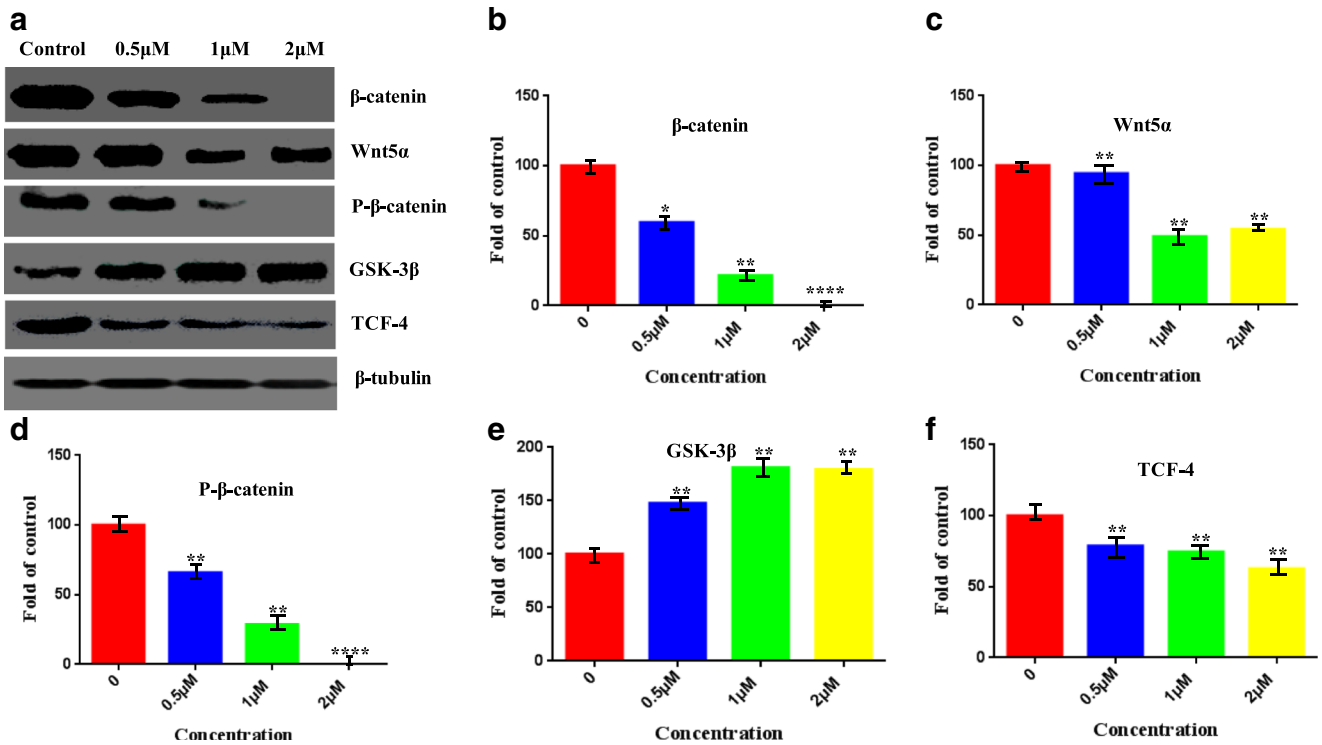


Fig. 6 Derivative 10 regulated Wnt/β-Catenin pathway in SGC7901 cells. **: $p < 0.01$ verse control. ****: $p < 0.0001$ verse control

and TCF4 in HepG2 cells. As shown in Fig. 6, the expression levels of β-catenin, Wnt5α, phospho β-catenin, and TCF-4 were all decreased and the expression level of GSK-3β was increased in treated SGC7901 cells. All these results indicated that hybrid **10** inhibited SGC7901 cells growth and migration via regulating the Wnt/β-Catenin pathway.

Analogue 10 regulated the AKT/mTOR pathway in SGC7901 cells

The activation of AKT/mTOR pathway resulted in hyperactive signaling cascades related to cellular growth, proliferation, and migration [35]. In AKT/mTOR pathway, protein kinase B (also known as AKT) activation initiated a signal transduction cascade that promoted the cellular growth and proliferation [36]. In addition, mammalian target of rapamycin (mTOR) as an atypical serine/threonine protein kinase played an important role in the regulation of cellular growth and motility [37].

From Fig. 7, formononetin-coumarin hybrid **10** could downregulate the phosphorylation levels of AKT and mTOR in SGC7901 cells in a concentration-dependent manner. The tests on AKT/mTOR signaling pathway illustrated that formononetin-coumarin hybrid **10** might also regulate AKT/mTOR signaling pathway to inhibit growth and migration of SGC7901 cells.

Analogue 10 inhibited SGC7901 cells growth in vivo

To evaluate the antitumor effects of formononetin-coumarin hybrid **10** in vivo, a SGC7901 xenograft model was

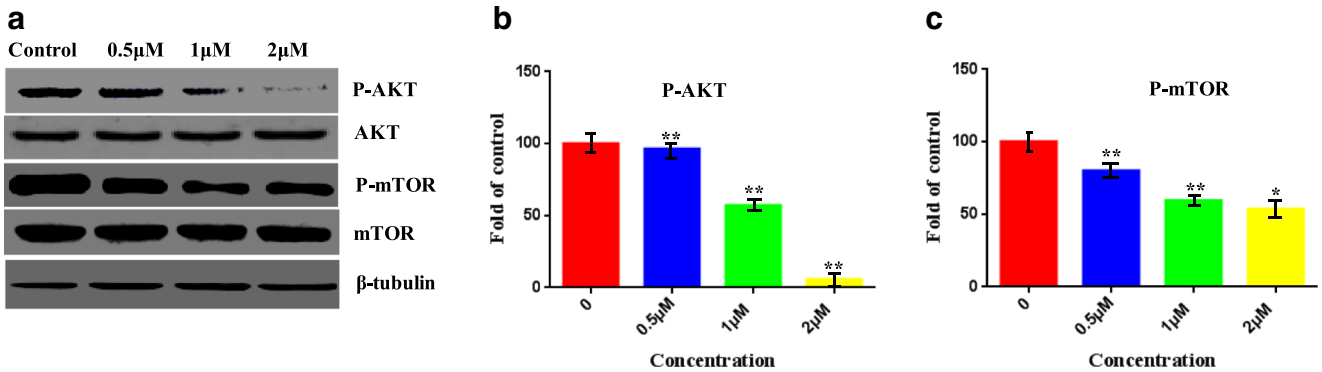


Fig. 7 Derivative 10 regulated AKT/mTOR pathway in SGC7901 cells. *: $p < 0.05$ verse control, **: $p < 0.01$ verse control

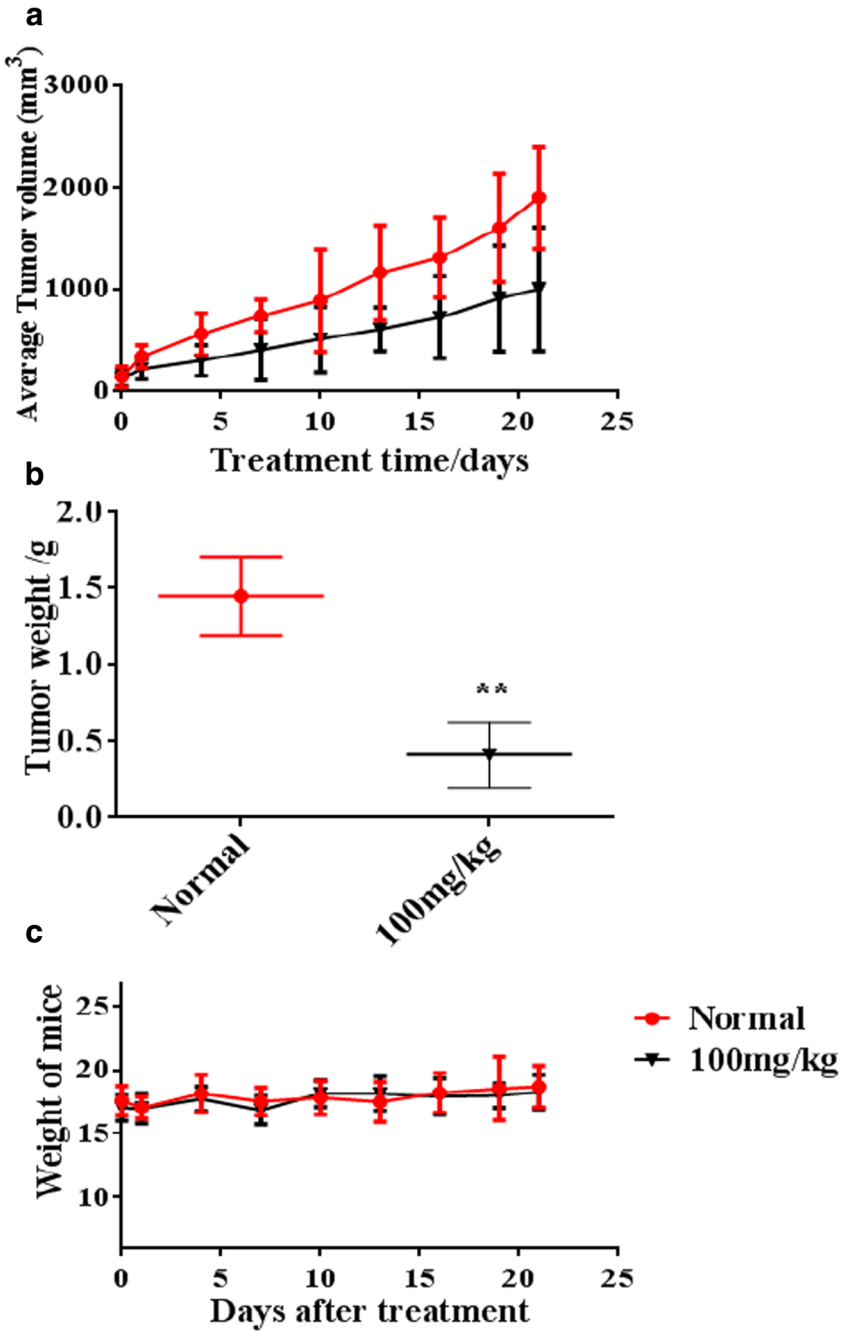


Fig. 8 **a** Average tumor volume (mm^3); **b** Tumor weight; **c** weight of mice. $^{**}P < 0.01$, significantly different compared with the control by test

established in nude mice by subcutaneously injecting SGC7901 cells. Mice were then randomly assigned to two groups (control and 100 mg/kg **10**) with 5 mice per group. The results in Fig. 8 showed that formononetin-coumarin hybrid **10** (100 mg/kg) caused a considerable suppression of tumor growth. The average tumor weights of control and formononetin-coumarin hybrid **10** groups were 1.45 ± 0.26 g and 0.41 ± 0.21 g (inhibitory rate: 71.72%), respectively. Importantly, the in vivo antitumor efficacy of formononetin-coumarin hybrid **10** was achieved without causing any obvious loss of body weight. These results suggested that formononetin-coumarin hybrid **10** has a low toxicity toward mice.

Discussion

We designed and synthesized a novel formononetin-coumarin hybrid by the molecular hybridization strategy based on the natural product formononetin. From this rational modification, formononetin-coumarin hybrid **10** displayed the more potent antiproliferative activity than formononetin against

MGC803 cells. Hybrid **10** was an novel SIRT1 inhibitor with an IC_{50} value of 2.52 μM by decreasing the expression of Sirt1 and increasing the expression of P53. In addition, formononetin-coumarin hybrid **10** could inhibit the migration against SGC7901 tumor cells via Wnt/β-Catenin and AKT/mTOR pathways. Importantly, formononetin-coumarin hybrid **10** potently inhibited tumor growth in vivo with a low toxicity toward mice.

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Author contributions Jian-Ning Yao, Xue-Xiu Zhang and Lian-Feng Zhang designed the research. Jian-Ning Yao, Xue-Xiu Zhang, Yan-Zhen Zhang, Jia-Heng Li, Dong-Yao Zhao, Bing Gao, Hai-Ning Zhou, Shi-Lin Gao, and Lian-Feng Zhang performed all the experiments. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Badgwell B, Blum M, Estrella J, Chiang Y-J, Das P, Matamoros A, Fournier K, Mansfield P, Ajani J (2015) Predictors of survival in patients with Resectable gastric Cancer treated with preoperative Chemoradiation therapy and gastrectomy. J Am Coll Surg 221(1): 83–90. https://doi.org/10.1016/j.jamcollsurg.2015.04.004
2. Sun H, Ni S, Ye M, Weng W, Zhang Q, Zhang M, Tan C, Wang L, Huang D, Du X, Xu M, Sheng W (2018) Hedgehog interacting protein 1 is a prognostic marker and suppresses cell metastasis in gastric Cancer. J Cancer 9(24):4642–4649. https://doi.org/10.7150/jca.27686
3. Chen X, Chen X, Zhang X, Wang L, Cao P, Rajamanickam V, Wu C, Zhou H, Cai Y, Liang G, Wang Y (2018) Curcuminoid B63 induces ROS-mediated paraptosis-like cell death by targeting TrxR1 in gastric cells. Redox Biol 21:101061–101061. https://doi.org/10.1016/j.redox.2018.11.019
4. Wang H, Zhang D, Ge M, Li Z, Jiang J, Li Y (2015) Formononetin inhibits enterovirus 71 replication by regulating COX- 2/PGE_2 expression. Virol J 12:35–35. https://doi.org/10.1186/s12985-015-0264-x
5. Li S, Dang Y, Zhou X, Huang B, Huang X, Zhang Z, Kwan YW, Chan SW, Leung GPH, Lee SMY, Hoi MPM (2015) Formononetin promotes angiogenesis through the estrogen receptor alpha-enhanced ROCK pathway. Sci Rep 5:16815–16815. https://doi.org/10.1038/srep16815
6. Wu D, Wu K, Zhu Q, Xiao W, Shan Q, Yan Z, Wu J, Deng B, Xue Y, Gong W, Lu G, Ding Y (2018) Formononetin administration ameliorates dextran sulfate sodium-induced acute colitis by inhibiting NLRP3 Inflammasome signaling pathway. Mediat Inflamm 2018:3048532–3048532. https://doi.org/10.1155/2018/3048532
7. Chen J, Zeng J, Xin M, Huang W, Chen X (2011) Formononetin induces cell cycle arrest of human breast Cancer cells via IGF1/PI3K/Akt pathways in vitro and in vivo. Horm Metab Res 43(10):681–686. https://doi.org/10.1055/s-0031-1286306
8. Zhang X, Ni Q, Wang Y, Fan H, Li Y (2018) Synergistic anticancer effects of Formononetin and Temozolomide on glioma C6 cells. Biol Pharm Bull 41(8):1194–1202. https://doi.org/10.1248/bpb.b18-00002
9. Lee H, Lee D, Kang KS, Song JH, Choi Y-K (2018) Inhibition of intracellular ROS accumulation by Formononetin attenuates cisplatin-mediated apoptosis in LLC-PK1 cells. Int J Mol Sci 19(3):813. https://doi.org/10.3390/ijms19030813
10. Hwang JS, Kang ES, Han SG, Lim D-S, Paek KS, Lee C-H, Seo HG (2018) Formononetin inhibits lipopolysaccharide-induced release of high mobility group box 1 by upregulating SIRT1 in a PPARδ-dependent manner. Peer J 6:e4208–e4208. https://doi.org/10.7717/peerj.4208
11. Yang Y, Zhao Y, Ai X, Cheng B, Lu S (2014) Formononetin suppresses the proliferation of human non-small cell lung cancer through induction of cell cycle arrest and apoptosis. Int J Clin Exp Pathol 7(12):8453–8461
12. Madadi NR, Penthala NR, Howk K, Ketkar A, Eoff RL, Borrelli MJ, Crooks PA (2015) Synthesis and biological evaluation of novel 4,5-disubstituted 2H-1,2,3-triazoles as cis-constrained analogues of combretastatin A-4. Eur J Med Chem 103:123–132. https://doi.org/10.1016/j.ejmech.2015.08.041
13. Xu G, Shi C, Guo D, Wang L, Ling Y, Han X, Luo J (2015) Functional-segregated coumarin-containing telodendrimer nanocarriers for efficient delivery of SN-38 for colon cancer treatment. Acta Biomater 21:85–98. https://doi.org/10.1016/j.actbio.2015.04.021
14. Khan S, Malla AM, Zafar A, Naseem I (2017) Synthesis of novel coumarin nucleus-based DPA drug-like molecular entity: in vitro DNA/Cu(II) binding, DNA cleavage and pro-oxidant mechanism for anticancer action. PLoS One 12(8):e0181783–e0181783. https://doi.org/10.1371/journal.pone.0181783
15. Zhang R-R, Liu J, Zhang Y, Hou M-Q, Zhang M-Z, Zhou F, Zhang W-H (2016) Microwave-assisted synthesis and antifungal activity of novel coumarin derivatives: Pyrano[3,2-c]chromene-2,5-diones. Eur J Med Chem 116:76–83. https://doi.org/10.1016/j.ejmech.2016.03.069
16. Yang H-L, Cai P, Liu Q-H, Yang X-L, Li F, Wang J, Wu J-J, Wang X-B, Kong L-Y (2017) Design, synthesis and evaluation of coumarin-pargyline hybrids as novel dual inhibitors of monoamine oxidases and amyloid-β aggregation for the treatment of Alzheimer's disease. Eur J Med Chem 138:715–728. https://doi.org/10.1016/j.ejmech.2017.07.008
17. Cao D, Liu Y, Yan W, Wang C, Bai P, Wang T, Tang M, Wang X, Yang Z, Ma B, Ma L, Lei L, Wang F, Xu B, Zhou Y, Yang T, Chen L (2016) Design, synthesis, and evaluation of in vitro and in vivo anticancer activity of 4-substituted Coumarins: a novel class of potent tubulin polymerization inhibitors. J Med Chem 59(12): 5721–5739. https://doi.org/10.1021/acs.jmedchem.6b00158
18. Kakwani MD, Suryavanshi P, Ray M, Rajan MGR, Majee S, Samad A, Devarajan P, Degani MS (2011) Design, synthesis and antimycobacterial activity of cinnamide derivatives: a molecular hybridization approach. Bioorg Med Chem Lett 21(7):1997–1999. https://doi.org/10.1016/j.bmcl.2011.02.022
19. Barbosa TP, Sousa SCO, Amorim FM, Rodrigues YKS, de Assis PAC, Caldas JPA, Oliveira MR, Vasconcellos MLAA (2011) Design, synthesis and antileishmanial in vitro activity of new series of chalcones-like compounds: a molecular hybridization approach. Bioorg Med Chem 19(14):4250–4256. https://doi.org/10.1016/j.bmc.2011.05.055

20. Wang Y, Zhou Y, Zheng Z, Li J, Yan Y, Wu W (2018) Sulforaphane metabolites reduce resistance to paclitaxel via microtubule disruption. Cell Death Dis 9(11):1134. https://doi.org/10.1038/s41419-018-1174-9
21. Deng L, Gao X, Liu B, He X, Xu J, Qiang J, Wu Q, Liu S (2018) NMT1 inhibition modulates breast cancer progression through stress-triggered JNK pathway. Cell Death Dis 9(12):1143. https://doi.org/10.1038/s41419-018-1201-x
22. Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M, Sinclair DA (2002) Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast Sir2 and human SIRT1. J Biol Chem 277(47):45099–45107
23. Feng Y, Wu J, Chen L, Luo C, Shen X, Chen K, Jiang H, Liu D (2009) A fluorometric assay of SIRT1 deacetylation activity through quantification of nicotinamide adenine dinucleotide. Anal Biochem 395(2):205–210. https://doi.org/10.1016/j.ab.2009.08.011
24. Sophia J, Kowshik J, Dwivedi A, Bhutia SK, Manavathi B, Mishra R, Nagini S (2018) Nimbolide, a neem limonoid inhibits cytoprotective autophagy to activate apoptosis via modulation of the PI3K/Akt/GSK-3β signalling pathway in oral cancer. Cell Death Dis 9(11):1087. https://doi.org/10.1038/s41419-018-1126-4
25. Rivera-Reyes A, Ye S, Marino GE, Egolf S, Ciotti G, Chor S, Liu Y, Posimo JM, PMC P, Pak K, Babichev Y, Sostre-Colón J, Tameire F, Leli NM, Koumenis C, Brady DC, Mancuso A, Weber K, Gladdy R, Qi J, Eisinger-Mathason TSK (2018) YAP1 enhances NF-κB-dependent and independent effects on clock-mediated unfolded protein responses and autophagy in sarcoma. Cell Death Dis 9(11):1108. https://doi.org/10.1038/s41419-018-1142-4
26. Zhao Y, Liu Y, Lin L, Huang Q, He W, Zhang S, Dong S, Wen Z, Rao J, Liao W, Shi M (2018) The lncRNA MACC1-AS1 promotes gastric cancer cell metabolic plasticity via AMPK/Lin28 mediated mRNA stability of MACC1. Mol Cancer 17(1):69. https://doi.org/10.1186/s12943-018-0820-2
27. Hwang ES, Song SB (2017) Nicotinamide is an inhibitor of SIRT1 in vitro, but can be a stimulator in cells. Cell Mol Life Sci 74(18):3347–3362. https://doi.org/10.1007/s00018-017-2527-8
28. Zhang W, Zhang Y, Guo X, Zeng Z, Wu J, Liu Y, He J, Wang R, Huang Q, Chen Z (2017) Sirt1 protects endothelial cells against LPS-induced barrier dysfunction. Oxidative Med Cell Longev 2017:4082102–4082102. https://doi.org/10.1155/2017/4082102
29. Liu D, Li S, Gong L, Yang Y, Han Y, Xie M, Zhang C (2018) Suppression of microRNA-141 suppressed p53 to protect against neural apoptosis in epilepsy by SIRT1 expression. J Cell Biochem. https://doi.org/10.1002/jcb.28216
30. Peck B, Chen C-Y, Ho K-K, Di Fruscia P, Myatt SS, Coombes RC, Fuchter MJ, Hsiao C-D, Lam EWF (2010) SIRT inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2. Mol Cancer Ther 9(4):844–855
31. Vaziri H, Dessain SK, Eaton EN, Imai S-I, Frye RA, Pandita TK, Guarente L, Weinberg RA (2001) hSIR2SIRT1 functions as an NAD-dependent p53 deacetylase. Cell 107(2):149–159. https://doi.org/10.1016/S0092-8674(01)00527-X
32. Maretzky T, Reiss K, Ludwig A, Buchholz J, Scholz F, Proksch E, de Strooper B, Hartmann D, Saftig P (2005) ADAM10 mediates E-cadherin shedding and regulates epithelial cell-cell adhesion, migration, and beta-catenin translocation. P Natl Acad Sci USA 102(26):9182–9187. https://doi.org/10.1073/pnas.0500918102
33. Cai C, Zhu X (2012) The Wnt/β-catenin pathway regulates self-renewal of cancer stem-like cells in human gastric cancer. Mol Med Rep 5(5):1191
34. Fodde R, Brabletz T (2007) Wnt/β-catenin signaling in cancer stemness and malignant behavior. Curr Opin Cell Biol 19(2):150–158. https://doi.org/10.1016/j.ceb.2007.02.007
35. Li H, Zhang B, Liu Y, Yin C (2014) EBP50 inhibits the migration and invasion of human breast cancer cells via LIMK/cofilin and the PI3K/Akt/mTOR/MMP signaling pathway. Med Oncol 31(9):162. https://doi.org/10.1007/s12032-014-0162-x
36. Li H, Zeng J, Shen K (2014) PI3K/AKT/mTOR signaling pathway as a therapeutic target for ovarian cancer. Arch Gynecol Obstet 290(6):1067–1078. https://doi.org/10.1007/s00404-014-3377-3
37. Demirci S, Doğan A, Apdik H, Tuysuz EC, Gulluoglu S, Bayrak OFS, Şahin F (2018) Cytoglobin inhibits migration through PI3K/AKT/mTOR pathway in fibroblast cells. Mol Cell Biochem 437(1):133–142. https://doi.org/10.1007/s11010-017-3101-2