Journal Pre-proofs

Synthesis of guggulsterone derivatives as potential anti-austerity agents against PANC-1 human pancreatic cancer cells

Aki Kohyama, Rei Yokoyama, Dya Fita Dibwe, Sahar El-Mekkawy, Meselhy R. Meselhy, Suresh Awale, Yuji Matsuya

PII: S0960-894X(20)30017-2
DOI: https://doi.org/10.1016/j.bmcl.2020.126964
Reference: BMCL 126964

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 2 December 2019
Revised Date: 8 January 2020
Accepted Date: 9 January 2020

Please cite this article as: Kohyama, A., Yokoyama, R., Dibwe, D.F., El-Mekkawy, S., Meselhy, M.R., Awale, S., Matsuya, Y., Synthesis of guggulsterone derivatives as potential anti-austerity agents against PANC-1 human pancreatic cancer cells, *Bioorganic & Medicinal Chemistry Letters* (2020), doi: https://doi.org/10.1016/j.bmcl.2020.126964

Synthesis of guggulsterone derivatives as potential anti-austerity agents against PANC-1 human pancreatic cancer cells

Aki Kohyama[a], Rei Yokoyama[a], Dya Fita Dibwe[b], Sahar El-Mekkawy,[c]

Meselhy R. Meselhy[d], Suresh Awale[*,b], Yuji Matsuya[*,a]

[a]*Faculty of Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan*

[b]*Division of Natural Drug Discovery, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan*

[c]*Department of Chemistry of Natural Compounds, National Research Centre, Elbohouth St., Giza, Egypt*

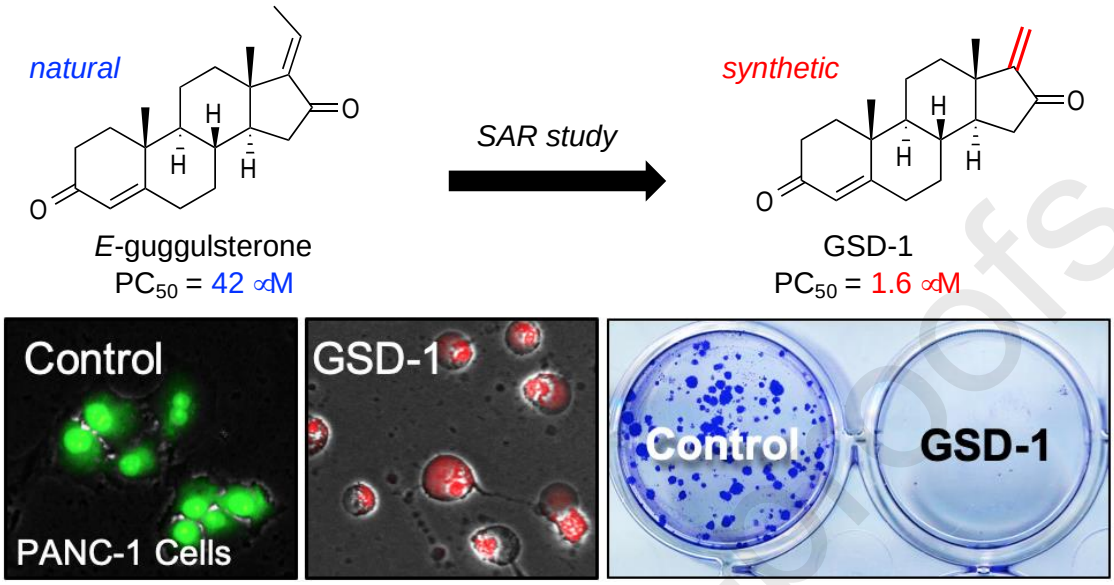
[d]*Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt*

* Corresponding Authors:
suresh@inm.u-toyama.ac.jp (Suresh Awale)
matsuya@pha.u-toyama.ac.jp (Yuji Matsuya)

E-and *Z*- guggulsterones and nine guggulsterone derivatives (GSDs) were synthesized and evaluated for their preferential cytotoxicity against human PANC-1 cell in nutrient deprived medium utilizing antiausterity strategy. Among the synthesized compounds, GSD-1 and GSD-7 showed potent cytotoxicity against PANC-1 cells under nutrient-deprived conditions in a concentration dependent manner, with a PC_{50} value of 1.6 μM and 3.2 μM, respectively. The effect of GSD-1 and GSD-7 was further evaluated in a real time using live cell imaging. Both of these compounds altered PANC-1 cell morphology, leading to cell death at sub micromolar concentration range. GSD-1 and GSD-7 also inhibited PANC-1 cell colony formation in a concentration-dependent manner. GSD-1 and GSD-7 are lead structure for the anti-austerity drug development.

Keywords: Guggulsterone; Pancreatic cancer; PANC-1; antiausterity agent; preferential cytotoxicity; drug discovery; steroid

(Graphical Abstract)



cause of cancer death.[1] The 5-year survival rate of the patients is the lowest among different cancer types because the patients are diagnosed usually at the advanced metastatic stage.[2] In addition, therapeutic protocols for the treatment of pancreatic cancer often fail to be effective due to drug resistance and impaired drug delivery pathways.[2c,2d,3] Therefore, novel chemotherapeutic agents to target pancreatic cancer are urgently required. An "anti-austerity strategy" has been developed[4,5] as a promising approach for drug discovery against pancreatic cancer. Human Pancreatic tumors are hypovascular in nature with limited supply of nutrients, the cancer cells are in starvation state within the tumor microenvironment. Therefore, tolerance to nutrition starvation is one of the critical factors for the pancreatic cancer cells to survive in tumor microenvironment. [4,5] It has been found that Human PANC-1 pancreatic cancer survives for over 3 days even in the complete absence of essential nutrients such as glucose, amino acids, and serum. Discovery of agents inhibiting such tolerance to starvation leading to cancer cell survival is the promising anti-austerity strategy in anti-cancer drug discovery.[6,7] In this approach, the compounds or extracts are exposed to pancreatic cancer cell line under the nutrient deprived medium (NDM) and the normal medium (DMEM). Compounds that show preferential cytotoxicity under NDM is selected as anti-austerity agents. The activity is represented as a PC_{50} value, which is the concentration at which 50% of the tumor cells were preferentially killed in NDM without exhibiting cytotoxicity in DMEM. This strategy has been successfully employed in the screening of thousands of medicinal plant extracts and the compounds for the discovery of unique anti-cancer agents from the natural source.[6,7] A natural lignan "arctigenin" has been discovered as one of the promising antiausterity agent, which has successfully passed early Phase II human clinical trial, with significant survival benefits among the pancreatic patients without toxic side effect.[8] It encouraged us to explore on natural product candidates to discover potent antiausterity agents through medicinal chemistry approach.

Commiphora mukul, a plant widely used in the Indian traditional medicine to treat various diseases.[9] It has been reported for anti-proliferative activity against various cancer cells,[10,11] therefore is an attractive candidate for cancer chemotherapy drug development. Meanwhile, there have been remarkable efforts in synthesizing potential anti-austerity agents.[12-14] In this project, we initiated a medicinal chemistry project and synthesized new GS derivatives (GSD) in an attempt to discover potential anti-austerity agents.

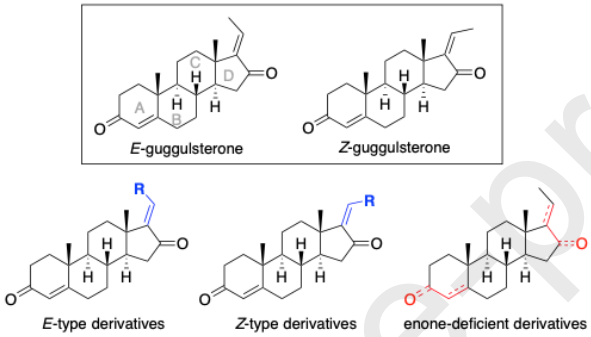
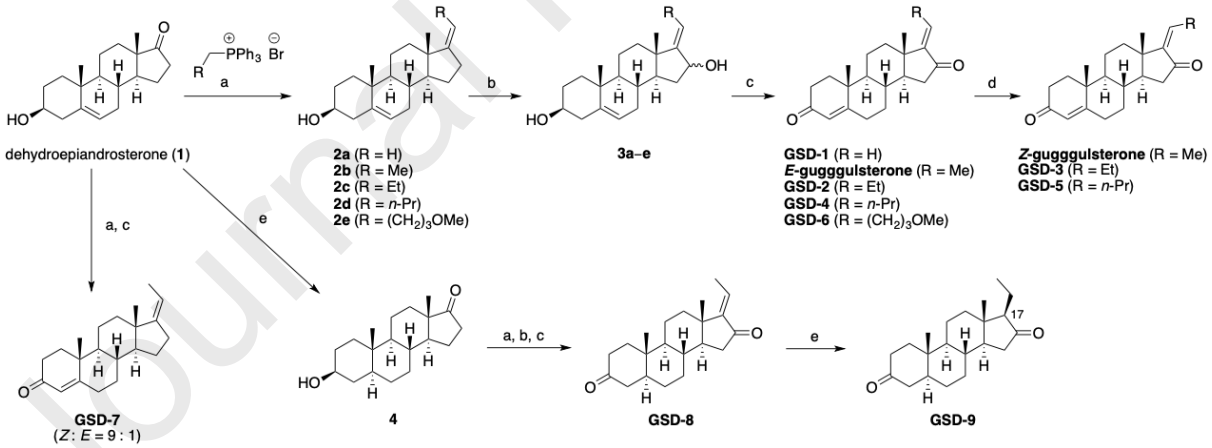


Figure 1. Molecular design of guggulsterone derivatives.



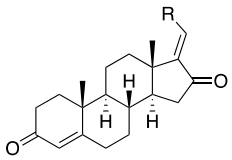
Scheme 1. Synthesis of guggulsterone derivatives

a. R-CH_2PPh_3Br (4 eq), *t*-BuOK (4 eq), THF, reflux, 1−24 h; b. SeO_2 (1 eq), TBHP (2.4 eq), CH_2Cl_2, 0 °C, 3−3.5 h; c. cyclohexanone (10 eq), Al(O*i*Pr)$_3$ (2.5 eq), benzene, 80 °C, 2−3 h 16−63% (3 steps); d. *p*-TsOH (0.1 eq), benzene, 80 °C, 1 h, 13−26%; e. Pd/C, H_2, EtOH, 2.5−6 h, 93−94%

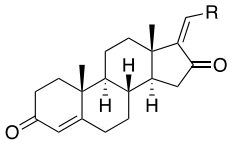
First, we designed the following guggulsterone derivatives; alkyl-substituted derivatives on D-ring (*E*-type), these (*Z*)-isomers (*Z*-type), and enone-deficient derivatives (Fig. 1). Generally, intrinsic electrophiles are reactive toward various intracellular molecules to play essential roles in cellular signals. In this context, two enone moieties on GS are expected to play crucial roles for the biological activity. Thus, these enone-deficient derivatives, as well as the side chain-modulated derivatives, were designed for the structure-activity-relationship (SAR) study.

For the concise and flexible synthesis of our designed GS derivatives in a short time, Kang's synthetic method was utilized.[15a,16] It is composed of Wittig reaction, allylic oxidation,[17] and Oppenauer reaction by using dehydroepiandrosterone (**1**) as a starting material. The use of various Wittig reagents enabled us to introduce different alkyl groups on D-ring (Scheme 1). The higher stereoselectivities were observed to give *E*-alkenes **2a-e** with trace amounts of *Z*-isomers. The resulting alkenes **2a-e** were oxidized to dienones via allylic oxidation and the subsequent Oppenauer reaction to provide all *E*-type derivatives, including natural *E*-GS. Furthermore, ***E*-GS**, **GSD-2**, and **GSD-4** were partially converted to *Z*-isomers under Kang's isomerization condition.[18] For the synthesis of enone-deficient derivatives, **GSD-7** was obtained as an inseparable mixture of *E*/*Z* isomer via Wittig reaction followed by Oppenauer reaction. Synthesis of **GSD-8** was achieved from the known compound **4** through the three-step sequence. Hydrogenation of **GSD-8** proceeded in a stereoselective manner to give **GSD-9** as a single diastereomer. Considering previous reports,[19] the reaction would proceed from sterically less hindered α-face, providing the 17-ethyl derivative with a β-configuration.

Table 1



E-type derivatives

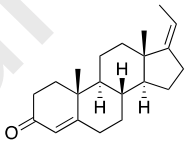


Z-type derivatives

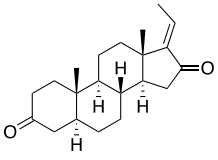
compound	*E*/*Z*	R	PC_{50} (μM) (NDM)	IC_{50} (μM) (DMEM)
E-guggulsterone	*E*	Me	42	> 100
GSD-1		H	1.6	11
GSD-2	*E*	Et	50	> 100
GSD-4	*E*	*n*-Pr	14	> 100
GSD-6	*E*	$(CH_2)_3OMe$	52	> 100
Z-guggulsterone	*Z*	Me	37	42
GSD-3	*Z*	Et	17	> 100
GSD-5	*Z*	*n*-Pr	18	> 100
arctigenin[a]			0.80	

[a]positive control

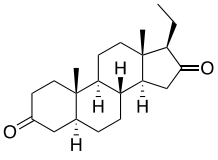
Table 2



GSD-7



GSD-8



GSD-9

compound	PC_{50} (μM) (NDM)	IC_{50} (μM) (DMEM)
E-guggulsterone	42	> 100
GSD-7	3.2	> 100
GSD-8	> 100	> 100
GSD-9	18	> 100

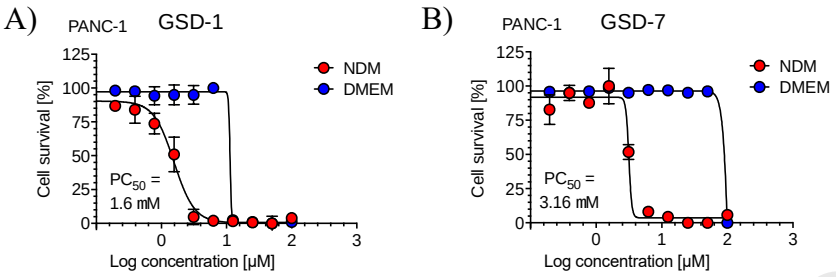


Figure 2. Preferential cytotoxic activity of A) GSD-1 and B) GSD-7 against the PANC-1 human pancreatic cancer cell line in nutrient-deprived medium (NDM) and Dulbecco's modified Eagle's medium (DMEM).

Following the antiausterity strategy, the synthesized *E*-GS, *Z*-GS, and nine GSDs were investigated for its anti-austerity activity against the PANC-1 tumor cell line (Table 1, 2, Figure 2). Among these, GSD-1 and GSD-7 showed the most potent preferential cytotoxicity against PANC-1 cells in NDM, exhibiting a PC_{50} value of 1.6 μM and 3.2 μM, respectively. In particular, the PC_{50} value of GSD-1 is comparable to that of arctigenin (PC_{50} 0.8 μM), a positive control used in this study.[8] The selectivity index of GSD-1 and GSD-7 in NDM was found to be 6.5 times and 34 times compared to DMEM. Based on the observed activity, a clear structure and activity can be deduced. In particular, table 1 shows that unsubstituted exocyclic double bond within enone moiety in D-ring of GSDs seems to be essential for the potent activity. (GSD-1 > *E* and *Z*-guggulsterone and GSD-2−6). All the GSD derivatives with substituents at exocyclic double bond displayed weak activity. Table 2 shows cytotoxicities of enone-deficient derivatives. **GSD-8** and **GSD-9** exhibited inactive or weak cytotoxicity. On the other hand, **GSD-7** showed potent preferential cytotoxicity with PC_{50} value of 3.2 μM, implying that one enone moiety on A-ring is important for cytotoxicity of **GSD-7**.

The promising antiausterity activity of GSD-1 and GSD-7 encouraged us to investigate the effect of these compounds on PANC-1 cells in a real-time frame. Therefore, PANC-1 cells

were treated with GSD-1 (3 µM) and GSD-7 (5 µM) in NDM, and incubated in a stage-top incubator at 37 °C in an atmosphere of 5% CO_2. The image of cellular events was captured in every 10 min in the phase-contrast mode on an EVOS FL digital cell imaging system for 24 h. As seen in Movie 1, GSD-1 and GSD-7 inhibited the cell mobility within short time after exposure leading to shrinkage of PANC-1 cells followed by swelling of cells and leakage of cytoplasmic contents into the media causing cell death within 12 h (Figure 3, movie 1).

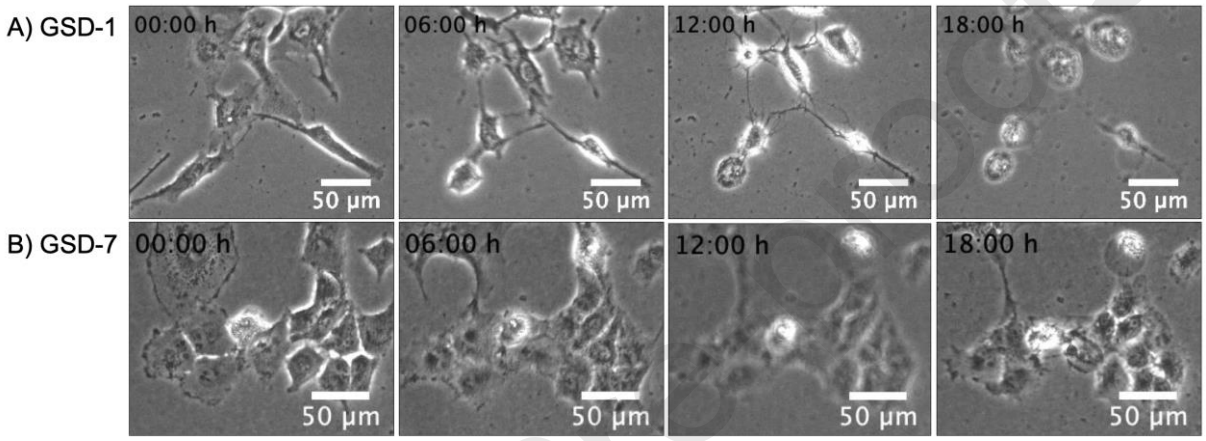
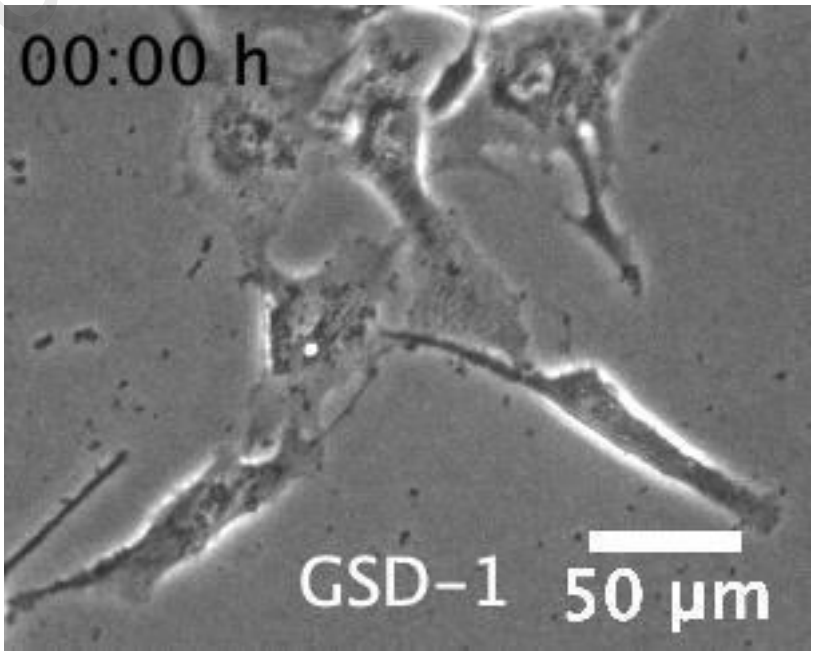


Figure 3. Captures of the live imaging of the effect of (A) GSD-1 (3 µM), and (B) GSD-7 (5 µM) on PANC-1 cells at different intervals of time (hour: minute) in nutrient deprived medium.

[Please insert **movie 1** here in the web version]

[Please insert this movie clip in the HTML version of the paper]



Movie 1. Time-lapse video showing the detailed events upon exposure to GSD-1 (3 µM) and GSD-7 (5 µM) against PANC-1 cells in nutrient-deprived medium (NDM) with one frame per 10 min for 24 h on the EVOS FL digital imaging system (10× objective)

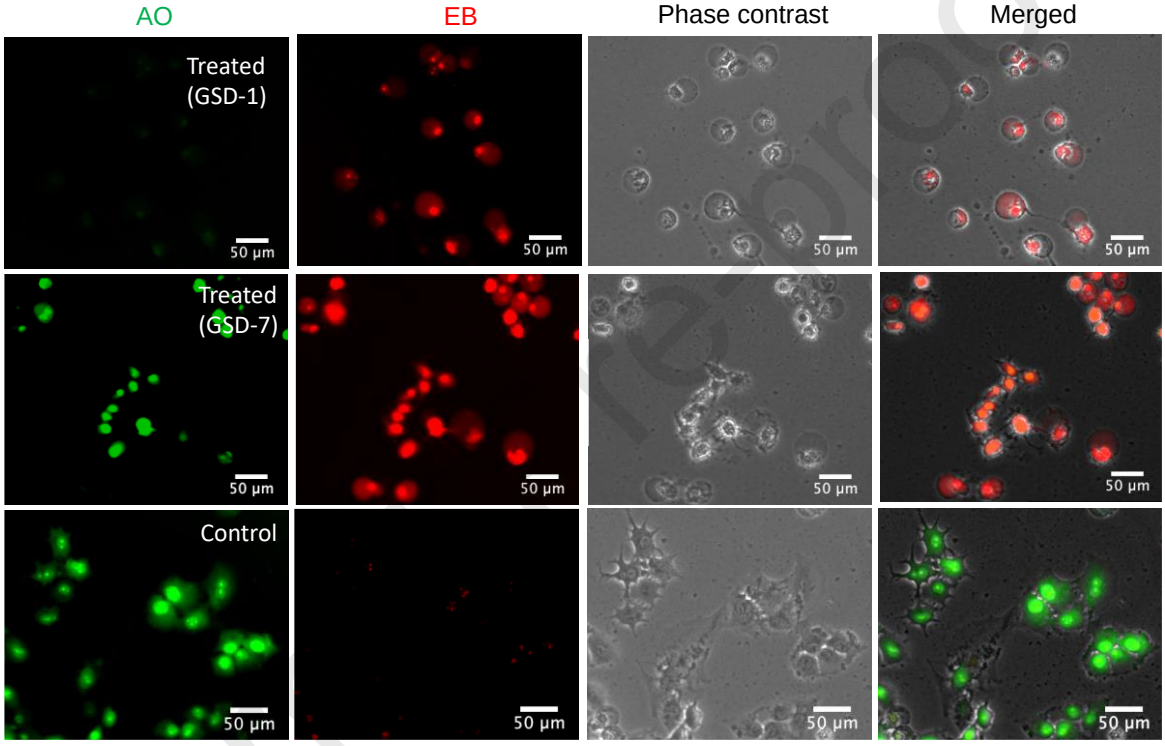


Figure 4. Morphological changes of PANC-1 cells induced by GSD-1 ((3 µM) and GSD-7 (5 µM), in comparison to untreated cells (i.e., the control) in nutrient-deprived medium (NDM). PANC-1 tumor cells were treated with GSD-1 and GSD-7 in NDM and incubated for 24 h. Cells were stained with ethidium bromide (EB) and acridine orange (AO) and photographed under fluorescence (red and green) and phase contrast modes using an EVOS FL digital microscope.

The effect of GSD-1 and GSD-7 on the alteration of PANC-1 morphology and cell death was further visualized by the ethidium bromide (EB) – acridine orange (AO) double-staining fluorescence assay. In this assay, the live cell emits bright-green fluorescence due to AO, while dead or dying cells and emits predominantly red fluorescence due to EB. PANC-1 cells

in the control show an intact cellular morphology and emitted exclusive green fluorescence of live cells even after exposed to nutrient deprivation for 24 hours. Contrary to this, PANC-1 cells treated with GSD-1 and GSD-7 showed rounded cell morphology of PANC-1 cells' and emitted exclusive red fluorescence, suggestive of total cell death.

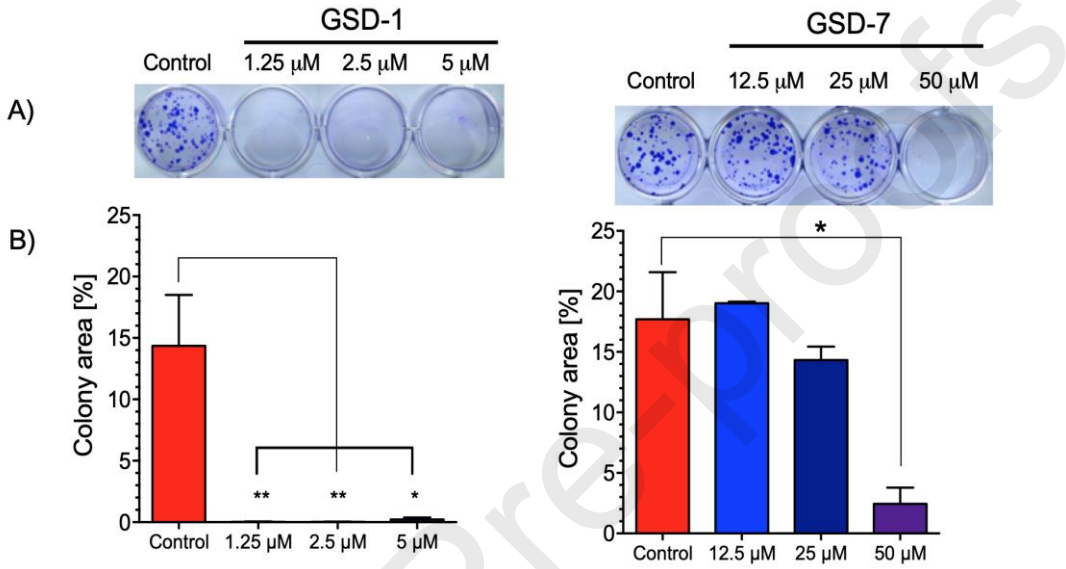


Figure 5. Effect of GSD-1 and GSD-7 on colony formation by PANC-1 cells. (A) Representative PANC-1 cell colonies treated with different concentrations of GSD-1 and GSD-7. (B) Graph showing mean values of the area occupied by PANC-1 cell colonies (three replicates). ****$p < 0.0001$, *$p < 0.05$ when compared with the untreated control group.

All these promising biological responses of GSD-1 and GSD-7 encouraged us to further evaluate these compounds against colony formation potential of PANC-1 cells. Pancreatic cancer is highly metastatic, and the invading cancer cells form metastatic colonies in the distant organs such as stomach, liver and lungs. Therefore, antiausterity agents with inhibitory activity against colony formation are therapeutically beneficial against pancreatic cancer. Hence, GSD-1 and GSD-7 was investigated against PANC-1 cell colony formation following the protocol described previously. PANC-1 cells (500 cells) were exposed to GSD-1 (1.25, 2.5 and 5.0 μM) and GSD-7 (12.5, 25 and 50 μM) at non-cytotoxic concentration in DMEM and incubated for

24 h. After that, the cells were washed twice with PBS followed by addition of fresh DMEM (without any test compound) and placed in a CO_2 incubator to allow colony formation for 10 days. At the end of experiment, cells were stained with crystal violet, and the area occupied by colony is evaluated. As shown in the Figure 5, the control PANC-1 cells grew and occupied 14%-18% of the total well area. Contrary to that, PANC-1 cells exposed with GSD-1 showed complete inhibition of colony formation even at the lowest tested dose of 1.25 μM. GSD-7 on the other hand inhibited colony formation only at the maximum tested concentration of 50 μM. From these results, GSD-1 was concluded as the most rewarding candidate for the drug development against pancreatic cancer. During nutrition starvation, Akt is overexpressed in pancreatic tumor cells and therefore regarded as austerity marker. Antiausterity agents, such as arctigenin, grandifloracin, kigamycin, ancistrolikokine E_3 have been found to inhibit Akt activation.[4a,5,7a] Therefore, it is possible that GSD derivatives might inhibit the activation of the Akt pathway leading to preferential cytotoxicity during the starvation condition. Further works on this area is currently in progress and will report in due course.

■ ACKNOWLEDGMENTS

This work was supported by a grant from the Japanese Society for the Promotion of Science (JSPS Kakenhi #16K08319) and Kobayashi International Scholarship Foundation to S.A., JSPS Core-to-Core Program (B. Asia-Africa Science Platforms) to Y.M., and Tamura Science and Technology Foundation to A.K.

Supplementary data

Supplementary data containing analytical data and NMR spectral data of the synthesized compounds and experimental procedures.

Reference and note

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 2018;68:394-424.

2. (a) Ansari D, Tingstedt B, Andersson B, Holmquist F, Sturesson C, Williamsson C, Sasor A, Borg D, Bauden M, Andersson R. Pancreatic cancer: yesterday, today and tomorrow. Future Oncol. 2016;12:1929–1946. (b) Hidalgo M, Pancreatic cancer. N. Engl. J. Med. 2010;362: 1605–1617. (c) Teague A, Lim KH, Wang-Gillam A. Advanced pancreatic adenocarcinoma: a review of current treatment strategies and developing therapies. Ther. Adv. Med. Oncol. 2015:7:68–84. (d) Garrido-Laguna I, Hidalgo M. Pancreatic cancer: from state-of-the-art treatments to promising novel therapies. Nat. Rev. Clin. Oncol. 2015**;**12:319–334.

3. Long J, Zhang Y, Yu X, Yang J, LeBrun DG, Chen C, Yao Q, Li M. Overcoming drug resistance in pancreatic cancer. Expert Opin. Ther. Targets 2011**;**15:817–828.

4. (a) Awale S, Lu J, Kalauni SK, Kurashima Y, Tezuka Y, Kadota S, Esumi H. Identification of arctigenin as an antitumor agent having the ability to eliminate the tolerance of cancer cells to nutrient starvation. Cancer Res. 2006**;**66:1751-1757. (b) Awale S, Nakashima EMN, Kalauni SK, Tezuka Y, Kurashima Y, Lu J, Esumi H, Kadota S. Angelmarin, a novel anti-cancer agent able to eliminate the tolerance of cancer cells to nutrient starvation. Bioorg. Med. Chem. Lett. 2006;16:581–583.

5. Izuishi K, Kato K, Ogura T, Kinoshita T, Esumi H. Remarkable tolerance of tumor cells to nutrient deprivation: possible new biochemical target for cancer therapy. Cancer Res. 2000;60:6201–6207.

6. (a) Awale S, Ueda J, Athikomkulchai S, Abdelhamed S, Yokoyama S, Saiki I, Miyatake R. Antiausterity Agents from *Uvaria dac* and Their Preferential Cytotoxic Activity against Human Pancreatic Cancer Cell Lines in a Nutrient-Deprived Condition. *J Nat Prod*. 2012;75:1177−1183. (b) Awale S, Ueda J, Athikomkulchai S, Dibwe DF, Abdelhamed S, Yokoyama S, Saiki I, Miyatake R. Uvaridacols E-H, highly oxygenated antiausterity agents from *Uvaria dac*. J Nat Prod. 2012;75:1999-2002. (c) Awale S, Tawila AM, Dibwe DF,

Ueda JY, Sun S, Athikomkulchai S, Balachandran C, Saiki I, Matsumoto K, Esumi H. Highly oxygenated antiausterity agents from the leaves of *Uvaria dac.* Bioorg Med Chem Lett. 2017;27:1967−1971. (d) Nguyen HX, Nguyen MTT, Nguyen NT, Awale S. Chemical Constituents of Propolis from Vietnamese *Trigona minor* and Their Antiausterity Activity against the PANC-1 Human Pancreatic Cancer Cell Line. J Nat Prod. 2017;80:2345−2352. e) Nguyen HX, Do TNV, Le TH, , Nguyen MT, Nguyen NT, Esumi H, Awale S. Chemical constituents of *Mangifera indica* and their antiausterity activity against the PANC-1 human pancreatic cancer cell line. J Nat Prod. 2016;79:2053−2059. (f) Nguyen HX, Nguyen MTT, Nguyen TA, Nguyen NYT, Phan DAT, Thi PH, Nguyen THP, Dang PH, Nguyen NT, Ueda JY, Awale S. Cleistanthane diterpenes from the seed of *Caesalpinia sappan* and their antiausterity activity against PANC-1 human pancreatic cancer cell line. Fitoterapia. 2013;91:148-153. (g)Nguyen HX, Nguyen NT, Dang PH, Thi HP, Nguyen MTT, Van CM, Dibwe DF, Ueda JY, Awale S. Cassane diterpenes from the seed kernels of *Caesalpinia sappan*. Phytochemistry 2016;122:286-293. (h) Nguyen KDH, Dang PH, Nguyen HX, Nguyen MTT, Awale S, Nguyen NT. Phytochemical and cytotoxic studies on the leaves of *Calotropis gigantea*. Bioorg Med Chem Lett. 2017;27:2902−2906. (i) Nguyen NT, Nguyen MTT, Nguyen HX, Dang PH, Dibwe DF, Esumi H, Awale S. Constituents of the rhizomes of *Boesenbergia pandurata* and their antiausterity activities against the PANC-1 human pancreatic cancer line. J Nat Prod. 2017;80:141−148. (j) Li F, Awale S, Zhang H, Tezuka Y, Esumi H, Kadota S. Chemical Constituents of Propolis from Myanmar and Their Preferential Cytotoxicity against a Human Pancreatic Cancer Cell Line. J Nat Prod. 2009;72:1283-1287.

7. (a) Awale S, Dibwe DF, Balachandran C, Fayez S, Feineis D, Lombe BK, Bringmann G. Ancistrolikokine E3, a 5,8′-Coupled Naphthylisoquinoline Alkaloid, Eliminates the Tolerance of Cancer Cells to Nutrition Starvation by Inhibition of the Akt/mTOR/Autophagy Signaling Pathway. J. Nat. Prod. 2018;81:2282−2291. (b) Muyisa S, Lombe BK, Feineis D, Dibwe DF, Maharaj V, Awale S, Bringmann G. Ancistroyafungines A-D, 5,8′- and 5,1′ coupled naphthylisoquinoline alkaloids from a Congolese *Ancistrocladus* species, with antiausterity activities against human PANC-1 pancreatic cancer cells. Fitoterapia, 2018; 130: 6−16. (c) Lombe BK, Feineis D, Mudogo V, Brun R, Awale S, Bringmann G. Michellamines A6 and A7, and further mono- and dimeric naphthylisoquinoline alkaloids from a Congolese Ancistrocladus liana and their antiausterity activities against pancreatic cancer cells. RSC Adv. 2018;8:5243−5254. (d) Fayez S, Feineis D, Mudogo V, Awale S, Bringmann G. Ancistrolikokines E‐H and related 5,8′-coupled naphthylisoquinoline

alkaloids from the Congolese liana Ancistrocladus likoko with antiausterity activities against PANC-1 human pancreatic cancer cells. RSC Adv. 2017;7:53740−53751.

8. (a) Strimpakos AS, Saif MW. Update on phase I studies in advanced pancreatic adenocarcinoma. Hunting in darkness? JOP 2013**;**14:354–358. (b) Ikeda M, Sato A, Mochizuki N, Toyosaki K, Miyoshi C, Fujioka R, Mitsunaga S, Ohno I, Hashimoto Y, Takahashi H, Hasegawa H, Nomura S, Takahashi R, Yomoda S, Tsuchihara K, Kishino S, Esumi H. Phase I trial of GBS-01 for advanced pancreatic cancer refractory to gemcitabine. Cancer Sci. 2016;107:1818–1824.
9. (a) Benn WR, Dodson RM. The Synthesis and Stereochemistry of Isomeric 16-Hydroxy-17(20)-pregnenes. J Org. Chem. 1964;29:1142–1148. (b) Patil VD, Nayak UR, Dev S. Chemistry of Ayurvedic crude drugs—I. Tetrahedron 1972;28:2341–2352. (c) Sarkhel S, Yadava U, Prakas P, Jain GK, Singh S, Maulik PR. Guggulsterone E, a lipid-lowering agent from Commiphora mukul. Acta Crystallographica Section E Structure Reports Online 2001**;**57:o285–o286. (d) Gupta VK, Bandhoria P, Gupta BD, Gupta K. Crystal Structure of Guggulsterone Z. Crystallography Reports 2006;51:265–270.
10. (a) Singh SV, Zeng Y, Xiao D, Vogel VG, Nelson JB, Dhir R, Tripathi YB. Caspase-dependent apoptosis induction by guggulsterone, a constituent of Ayurvedic medicinal plant Commiphora mukul, in PC-3 human prostate cancer cells is mediated by Bax and Bak. Mol. Cancer Ther. 2005**;**4:1747–1754. (b) Shishodia S, Sethi G, Ahn KS, Aggarwal BB. Guggulsterone inhibits tumor cell proliferation, induces S-phase arrest, and promotes apoptosis through activation of c-Jun N-terminal kinase, suppression of Akt pathway, and downregulation of antiapoptotic gene products. Biochem. Pharmacol. 2007;74:118–130.
11. Macha MA, Rachagani S, Gupta S, Pai P, Ponnusamy MP, Batra SK, Jain M. Guggulsterone decreases proliferation and metastatic behavior of pancreatic cancer cells by modulating JAK/STAT and Src/FAK signaling. Cancer Lett. 2013;341:166–177.

12. (a) Magolan J, Coster MJ. J. Org. Chem. 2009;74:5083–5086. (b) Magolan J, Adams NBP, Onozuka H, Hungerford NL, Esumi H, Coster MJ. Synthesis and Evaluation of Anticancer Natural Product Analogues Based on Angelmarin: Targeting the Tolerance towards Nutrient Deprivation. ChemMedChem. 2012;7:766–770. (c) Jiang H, Hamada Y. nantioselective synthesis of angelmarin. Org. Biomol. Chem. 2009;7: 4173–4176. (d) Harris E, Banwell M, Willis A. Tetrahedron Lett. 2011;52:6887–6889.

13. (a) Awale S, Okada T, Dibwe DF, Maruyama T, Takahara S, Okada T, Endo S, Toyooka N. Design and Synthesis of Functionalized Coumarins as Potential Anti-austerity Agents that Eliminates Cancer Cell's Tolerance to Nutrition Starvation. Bioorg. Med. Chem. Lett. 2019;29:1779–1784. (b) Kudou N, Taniguchi A, Sugimoto K, Matsuya Y, Kawasaki M, Toyooka N, Miyoshi C, Awale S, Dibwe DF, Esumi H, Kadota S, Tezuka Y. Synthesis and antitumor evaluation of arctigenin derivatives based on antiausterity strategy. Eur. J. Med. Chem. 2013;60:76-88.

14. Devji T, Reddy C, Woo C, Awale S, Kadota S, Carrico-Moniz D. Pancreatic anticancer activity of a novel geranylgeranylated coumarin derivative. Bioorg. Med. Chem. Lett. 2011;21:5770–5773.

15. (a) Lee D, Kim T, Kim KH, Ham J, Jang TS, Kang KS, Lee JW. Evaluation of guggulsterone derivatives as novel kidney cell protective agents against cisplatin-induced nephrotoxicity. Bioorg. Med. Chem. Lett. 2017; 27:3156–3161. (b) Kang SJ, Kim JM, Koh SJ, Kim SH, Im JP, Jung HC, Kim JS. The guggulsterone derivative GG-52 inhibits NF-kappaB signaling in bone marrow-derived dendritic cells and attenuates colitis in IL-10 knockout mice. Life Sci. 2013;92:1064–1071. (c) Kim JM, Kang HW, Cha MY, Yoo D, Kim N, Kim IK, Ku J, Kim S, Ma SH, Jung HC, Song IS, Kim JS. Novel guggulsterone derivative GG-52 inhibits NF-kappaB signaling in intestinal epithelial cells and attenuates acute murine colitis. Lab. Invest. 2010;90:1004–1015.

16. Kang H, Ham J, Chin J. Process for preparing guggulsterones and guggulsterol. *WO 2004/094450A1* 2004.

17. Snider BB, Shi B. Syntheses of (E)- and (Z)-volkendousin. Tetrahedron 1999; 55, 14823-14828.

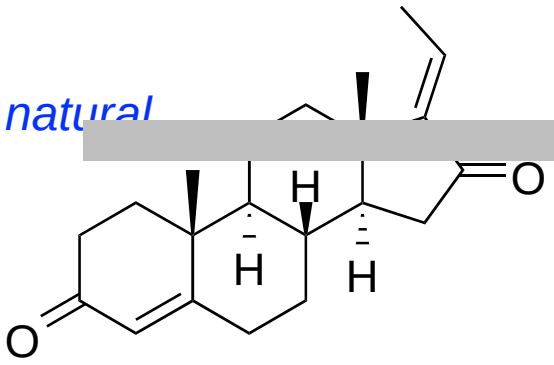
18. Ham J, Chin J, Kang H. A Regioselective Synthesis of E-Guggulsterone. Molecules 2011; 16: 4165–4171.

19. (a) Casimiro-Garcia A, De Clercq E, Pannecouque C, Witvrouw M, Loftus TL, Turpin JA, Buckheit RW, Fanwick PE, Cushman M. Synthesis and anti-HIV activity of cosalane analogues incorporating two dichlorodisalicylmethane pharmacophore fragments. Bioorg. Med. Chem. 2001;9:2827–2841. (b) Norden S, Bender M, Rullkötter J, Christoffers J. Androstanes with Modified Carbon Skeletons. European J. Org. Chem. 2011;2011:4543–4550. (c) Pore V, Aher N, Gonnade R. Stereoselective Construction of Steroidal Side Chain from 16-Dehydropregnenolone Acetate. Synlett. 2009; 2009:2005–2009.

Declaration of interests

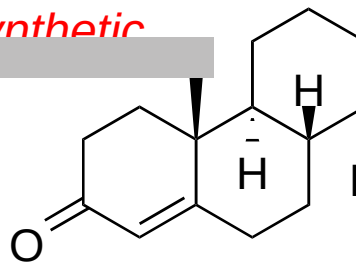
☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:



E-guggulsterone
PC_{50} = 42 μM

SAR study



GSD-1
PC_{50} = 1.6 μM