Chemical constituents and anti-pancreatic cancer activities of selected Thai medicinal plants

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Cancer continues to be a major health problem worldwide, accounting for nearly 10 million deaths in 2020. Among all types of cancer, pancreatic cancer is the deadliest, having the mortality rate nearly equivalent to the incidence rate. The incidence of pancreatic cancer continues to increase throughout the world, and is projected as the third leading cause of cancer death by 2025. Pancreatic cancer is mostly asymptomatic at the initial stage, and most pancreatic cancer patients are diagnosed in an advanced stage when the tumor has already metastasized to distant organs, making surgery almost impossible. Even the early-stage patients usually suffer from the disease recurrence within a year after surgical tumor removal. Chemotherapy is used to extend survival and/or relieve pancreatic cancer patients' symptoms. However, pancreatic tumors have an intrinsic resistance to almost all the clinically used chemotherapeutic agents. Therefore, there is an urgent need to find new effective agents against pancreatic cancer.

Pancreatic tumor in general shows hypovascularity with abundant fibrous stroma, leading to critical gradient of the nutrition supply within tumor microenvironment. However, pancreatic tumor cells have an inherent ability to adapt such stressed (austere) conditions by altering their energy metabolism, and gaining tolerance to nutrition starvation, a phenomenon known as "austerity" in cancer biology. The search of agents that inhibit cancer cells tolerance to nutrition starvation is a promising approach for the anticancer drug discovery.

In the present study, extracts of 24 selected Thai indigenous medicinal plants extracts were tested for their preferential cytotoxic activity (antiausterity activity) against PANC-1 human pancreatic cancer cell line in nutrient-deprived medium (NDM) and standard nutrient-rich medium (DMEM). Among the tested plants, five extracts exhibited promising activities. These include *Piper ribesoides* (PC_{50} = 24 μg/mL), *Citrus hystrix* (PC_{50} = 8.9 μg/mL), *Kaempferia parviflora* (PC_{50} = 3.3 μg/mL), *Derris scandens* (PC_{50} = 0.8 μg/mL), and *Boesenbergia pandurata* (PC_{50} = 0.5 μg/mL). The phytochemical investigation of these active extracts was carried out in order to identify the active constituents and elucidated the molecular mechanism.

1. Chemical constituents of *Kaempferia parviflora* and their antiausterity activity

Phytochemical investigation of *K. parviflora* extract led to the isolation of fourteen compounds, including two polyoxygenated cyclohexanes (**1** and **2**), eleven flavonoids (**3**–**13**), and *β*-sitosterol (**14**) (Chart 1). X-ray analysis was performed for compound **1** (Figure 1). All isolated compounds were tested for their preferential cytotoxicity against PANC-1 cells. Among them, 5-hydroxy-7-methoxyflavone (**3**) displayed the most potent activity with a PC_{50} value of 0.8 μM. It was also found to inhibit PANC-1 cancer cell colony formation in DMEM (Figure 2).[1]

2. Chemical constituents of *Citrus hystrix* and their antiausterity activity

Phytochemical investigation of *Citrus hystrix* extract led to the isolation of 10 coumarins (**15**−**24**) (Chart 2) including a new furanocoumarin named (*S*)-(−)-2′-methoxyoxypeucedanin hydrate (**15**). The absolute configuration of **15** was determined by comparing the $[\alpha]_D$ and ECD spectral data with those of (*R*)-(+)-oxypeucedanin hydrate (**17**) (Figure 3). All isolated compounds were tested for their preferential cytotoxicity against three human pancreatic cancer cell lines. Among these, bergamottin (**21**) was identified as the most active constituent. A real-time live cell imaging experiment revealed that compound **21** could induce cell shrinkage, membrane blebbing, and disintegration of organelles in PANC-1 cells. Bergamottin (**21**) was also found to inhibit PANC-1 cell migration and colony formation (Figure 4).[2]

3. Chemical constituents of *Derris scandens* and their antiausterity activity

Phytochemical investigation of *Derris scandens* extract led to the isolation of four prenylated isoflavones (**25**–**28**) (Chart 3) including a new compound named 4′-*O*-methylgrynullarin (**25**). The structure elucidation of the new compound was achieved by HRFABMS and NMR spectroscopic analysis (Figure 5). The isolated compounds exhibited potent antiausterity activity against PANC-1 human pancreatic cancer cell line under nutrient-deprived conditions. The new compound 4′-*O*-methylgrynullarin (**25**) was also found to inhibit PANC-1 cell migration and colony formation under nutrient-rich condition (Figure 6 and 7). Mechanistically, compound **25** inhibited key survival proteins in the Akt/mTOR signaling pathway in NDM (Figure 8).[3]

4. Chemical constituents of *Piper ribesoides* and their antiausterity activity

Phytochemical investigation of *Piper ribesoides* extract led to the isolation of six compounds (**14**, **29**−**33**) (Chart 4), including two new polyoxygenated cyclohexane derivatives, named ribesoidones A and B (**29** and **30**). The structural elucidation of the new compounds was achieved by a combination of HREIMS, NMR, and ECD spectroscopic analyses (Figure 9 and 10). Isolated compounds were tested for their antiausterity activity against PANC-1 human pancreatic cancer cell line. Among these, compounds **29**, **30** and **31** displayed potent preferential cytotoxic activity with PC_{50} values of 5.5–7.2 μM. Ribesoidone A (**29**) was also found to inhibit PANC-1 colony formation under normal nutrient-rich conditions (Figure 11).[4]

5. Chemical constituents of *Bosenbergia pandurata* and their antiausterity activity

Phytochemical investigation of *Bosenbergia pandurata* extract led to the isolation of five compounds (**34**−**37**) (Chart 5). All isolated compounds were tested for their preferential cytotoxicity against PANC-1 and MIA PaCa-2 human pancreatic cancer cell lines where most compounds exhibited potent activities (Table 1). (+)-Panduratin A (**34**) and geranyl-2,4-dihydroxy-6-phenethylbenzoate (**37**) were also found to inhibit PANC-1 cell migration (Figure 12) and colony formation in normal nutrient-rich condition (Figure 13 and 14). Mechanistically, these two compounds inhibited Akt/mTOR and autophagy signaling pathway, leading to selective PANC-1 cancer cell death under the nutrition starvation condition (Figure 15 and 16).[5,6] Moreover, (+)-isopanduratin A (**35**) was found to inhibit MIA PaCa-2 cell migration and colony formation in normal nutrient-rich condition. Mechanistically, it inhibited Akt/mTOR and autophagy survival signaling pathway in MIA PaCa-2 cells. (+)-Isopanduratin A (**35**) was also found to strongly suppress the MIA PaCa-2 tumor growth in a xenograft mouse model.[7]

6. Conclusion

Phytochemical investigation of five selected Thai medicinal plant extracts led to the isolation of **37** compounds including four new compounds. Among isolated compounds **3**, **21**, **25**, **29**, **34**, **35**, and **37** displayed the potent antiausterity activities. These compounds were studied for their anti-metastatic potential by employing a real-time cell migration and colony formation inhibition studies, and provided unbiased quantitative information of the effect of the tested compounds against pancreatic cancer cell motility, as evidenced by real-time movies. Mechanistically, compounds **25**, **34**, **35**, and **37** were found to inhibit the key survival proteins in the Akt/mTOR signaling pathway. In turn, isopanduratin A (**35**) was investigated for its *in vivo* anti-tumor activity against MIA PaCa-2 human pancreatic tumor xenograft in nude mice, and showed remarkable reduction of tumor over the period of 29 days study, with no toxicity at the administered dose. In summary, the current research led to the discovery of diverse natural product leads for the drug development against pancreatic cancer.

References

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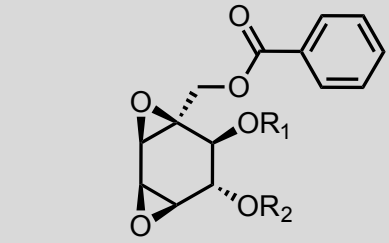
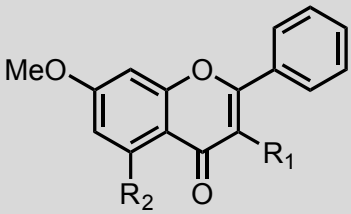
2. **Sijia Sun**, Ampai Phrutivorapongkul, Dya Fita Dibwe, Chandrasekar Balachandran and Suresh Awale. Chemical constituents of Thai *Citrus hystrix* and their antiausterity activity against the PANC-1 human pancreatic cancer cell line. *Journal of Natural Products*, **2018**, *81*, 1877–1883.
3. **Sijia Sun,** Dya Fita Dibwe, Min Jo Kim, Ashraf M. Omar, Nguyen Duy Phan, Haruka Fujino, Nusrin Pongterdsak, Kritsaya Chaithatwatthana, Ampai Phrutivorapongkul and Suresh Awale. A new anti-austerity agent, 4'-*O*-methylgrynullarin from *Derris scandens* induces PANC-1 human pancreatic cancer cell death under nutrition starvation *via* inhibition of Akt/mTOR pathway. *Bioorganic & Medicinal Chemistry Letters*, **2021**, *40*, 127967.
4. **Sijia Sun**, Ashraf M. Omar, Min Jo Kim, Nguyen Duy Phan, Yaowared Chulikhit and Suresh Awale. Chemical constituents of Thai *Piper ribesoides* and their antiausterity activities against the PANC-1 human pancreatic cancer cell line. *Fitoterapia*, **2021**, *151*, 104901.
5. **Sijia Sun**, Min Jo Kim, Ashraf M. Omar, Nguyen Duy Phan and Suresh Awale. (+)-Panduratin A induces PANC-1 human pancreatic cancer cell death preferentially under nutrient starvation by inhibiting PI3K/Akt/mTOR/autophagy signaling pathway. *Phytomedicine Plus*, **2021**, *1*(4),100101.
6. **Sijia Sun**, Min Jo Kim, Ashraf M. Omar, Nguyen Duy Phan, Mio Aoike and Suresh Awale. GDP induces PANC-1 human pancreatic cancer cell death preferentially under nutrient starvation by inhibiting PI3K/Akt/mTOR/Autophagy signaling pathway. *Chemistry and Biodiversity*, **2021**, *81*, e2100389.
7. **Sijia Sun**, Suresh Awale et al. *In vivo* anti-tumor effect of isopanduratin A against MIA PaCa-2 human pancreatic cancer xenograft model. **2021**. *In preparation.*

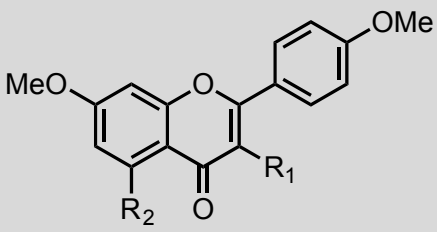
Chart 1. Structures of compounds isolated from *K. parviflora*



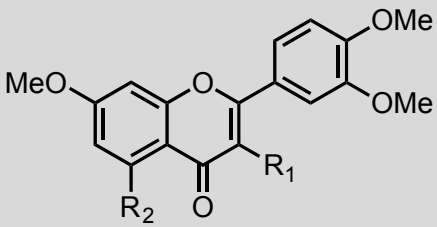
1 R_1 = H, R_2 = COMe
2 R_1 = COMe, R_2 = H



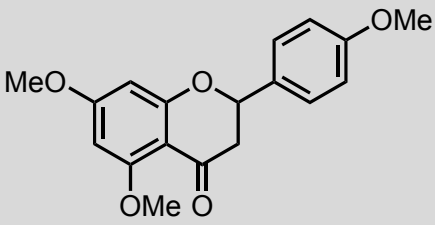
3 R_1 = H, R_2 = OH
4 R_1 = OMe, R_2 = OH
5 R_1 = H, R_2 = OMe
6 R_1 = OMe, R_2 = OMe



7 R_1 = H, R_2 = OH
8 R_1 = OMe, R_2 = OH
9 R_1 = H, R_2 = OMe
10 R_1 = OMe, R_2 = OMe



11 R_1 = OMe, R_2 = OH
12 R_1 = OMe, R_2 = OMe



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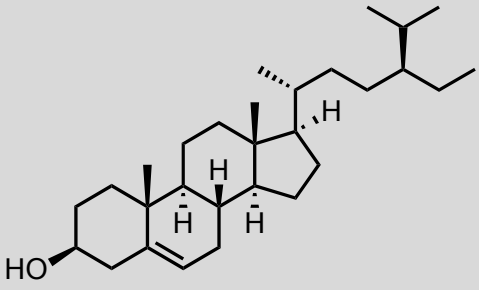

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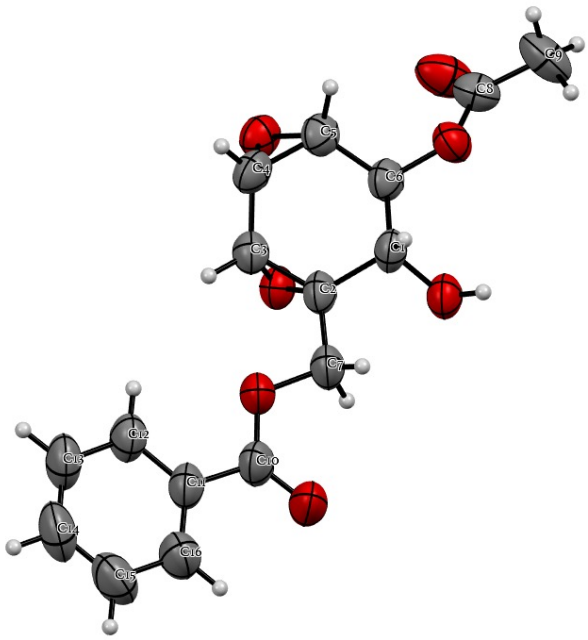


Figure 1. Anisotropic displacement ellipsoid plot of $C_{16}H_{16}O_7$ at the 70% probability level.

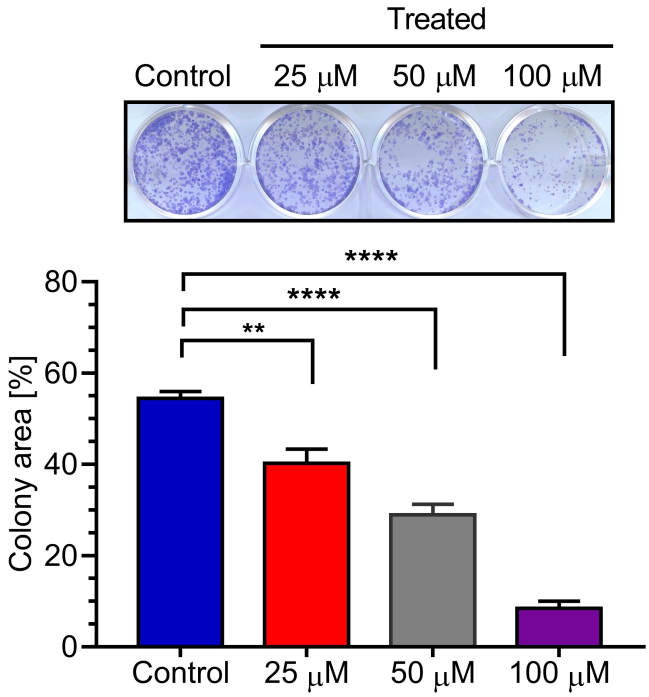
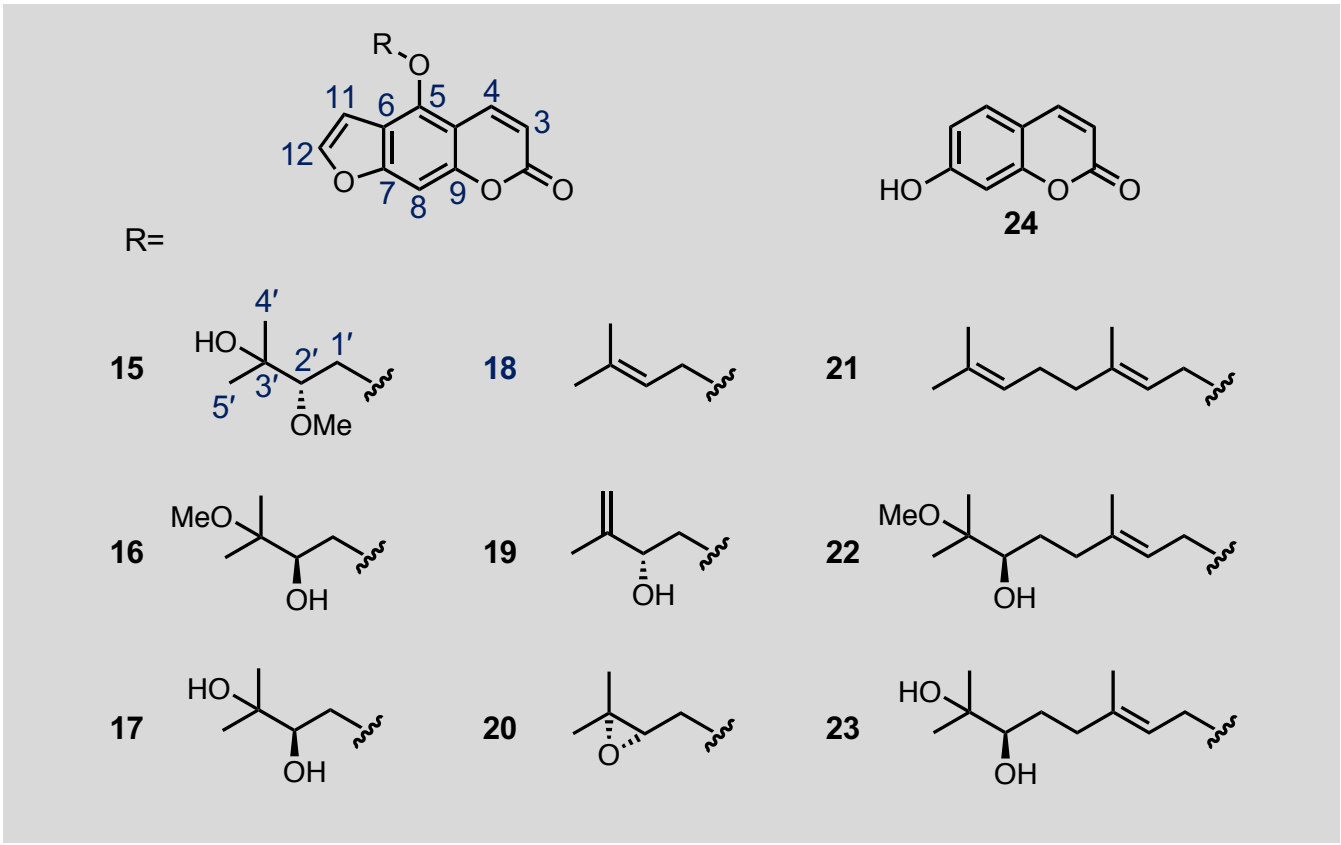


Figure 2. Effect of 5-hydroxy-7-methoxyflavone (**3**) on PANC-1 colony formation

Chart 2. Structures of compounds isolated from *C. hystrix*



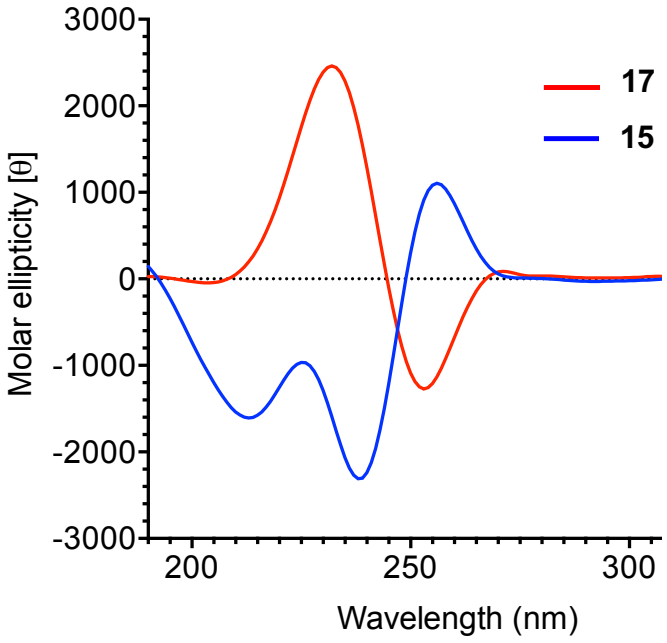


Figure 3. ECD spectra for compounds **15** and **17**.

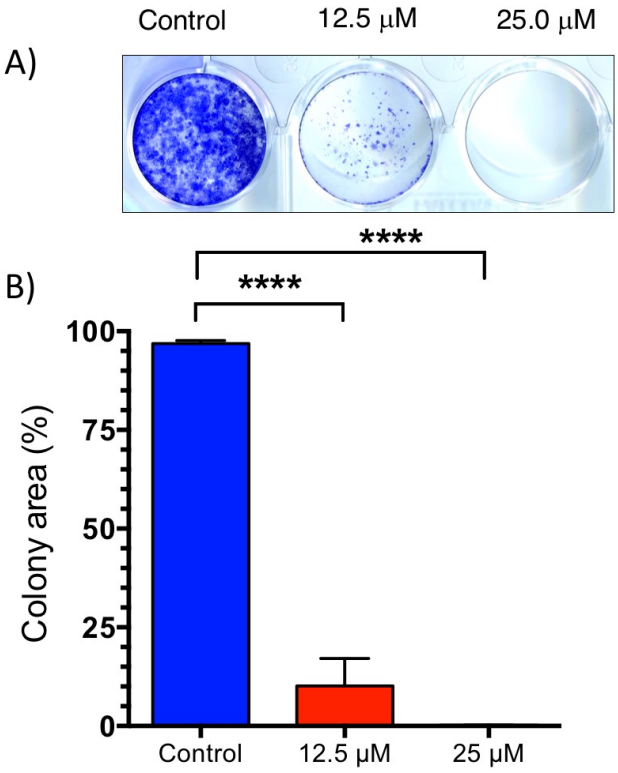
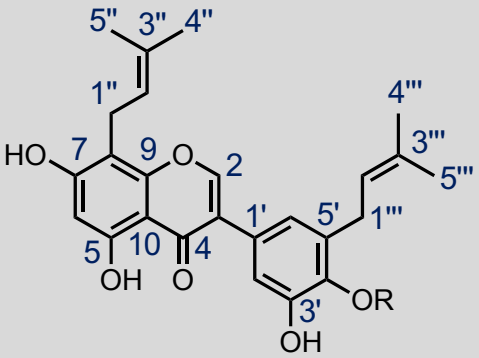


Figure 4. Effect of bergamottin (**21**) on PANC-1 cells colony formation.

Chart 3. Structures of compounds isolated from *D. scandens*



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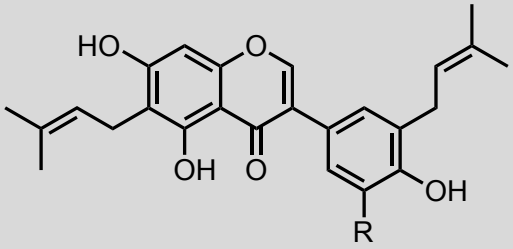


26 R = OH
27 R = H

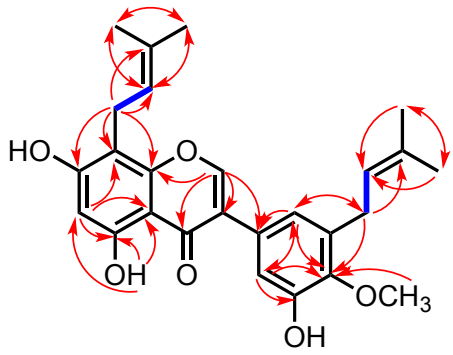


Figure 5. Connectivities (bold lines) deduced by the COSY and HMQC spectra and significant HMBC correlations (solid arrows) in **25**.

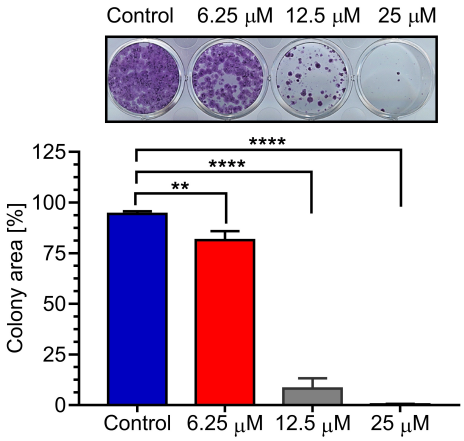


Figure 6. Effect of 4′-*O*-methylgrynullarin (**25**) on PANC-1 cells colony formation.

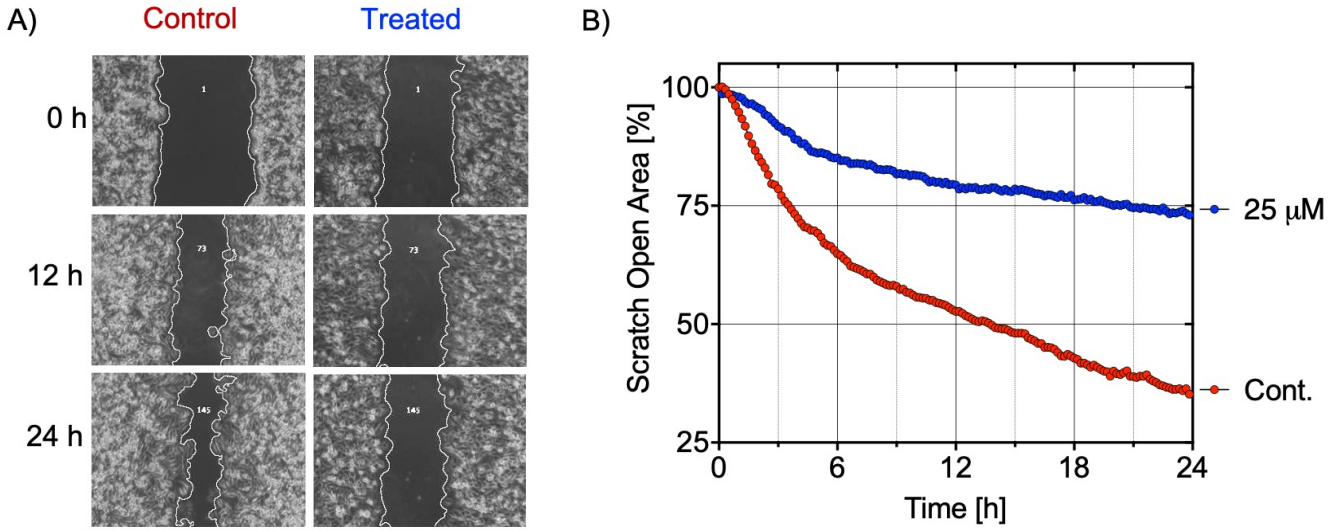


Figure 7. Compound **25** suppresses the migration of PANC-1 in a wound-healing assay in real-time.

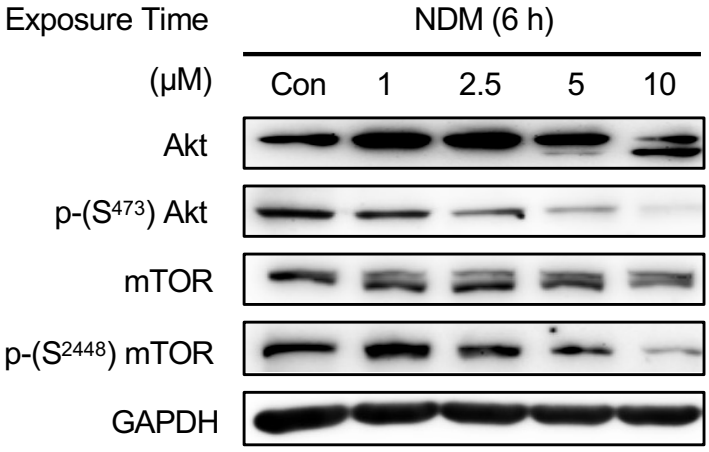
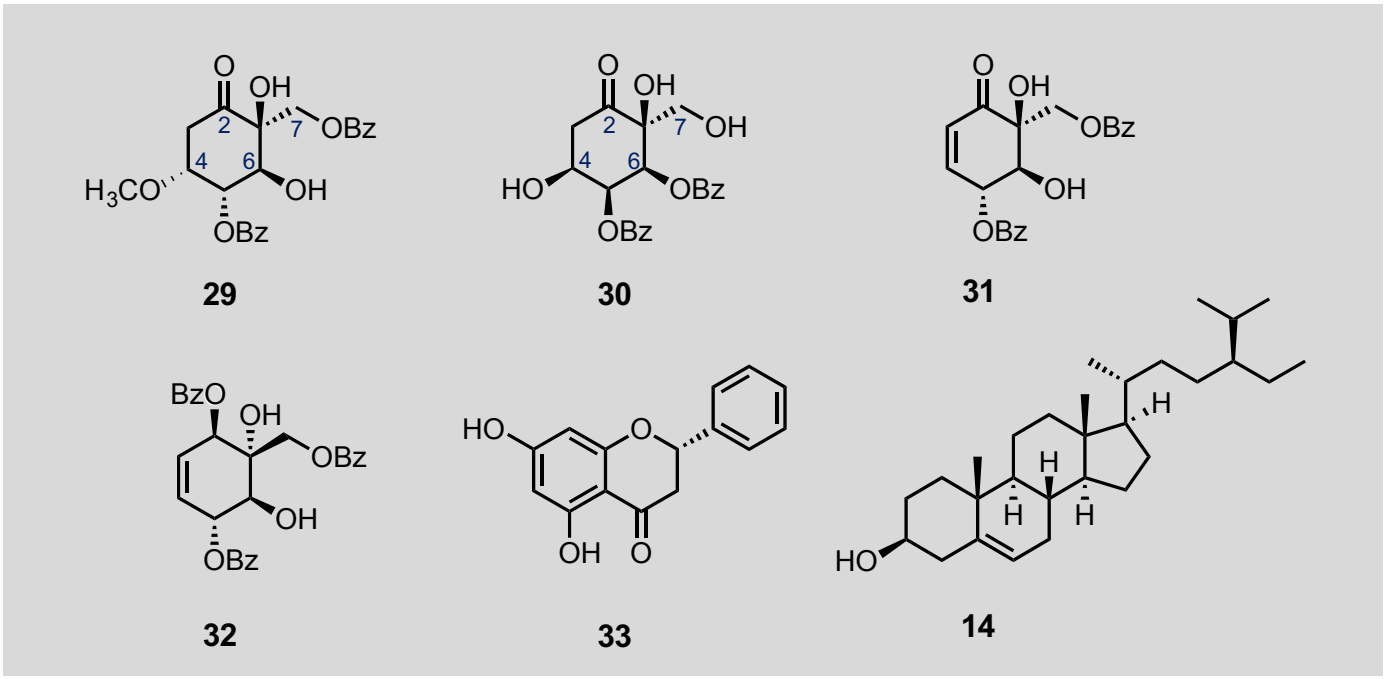


Figure 8. Effect of 4′-*O*-methylgrynullarin (**25**) on the key proteins involved in Akt/mTOR signaling in PANC-1 cells.

Chart 4. Structures of compounds isolated from *P. ribesoides*



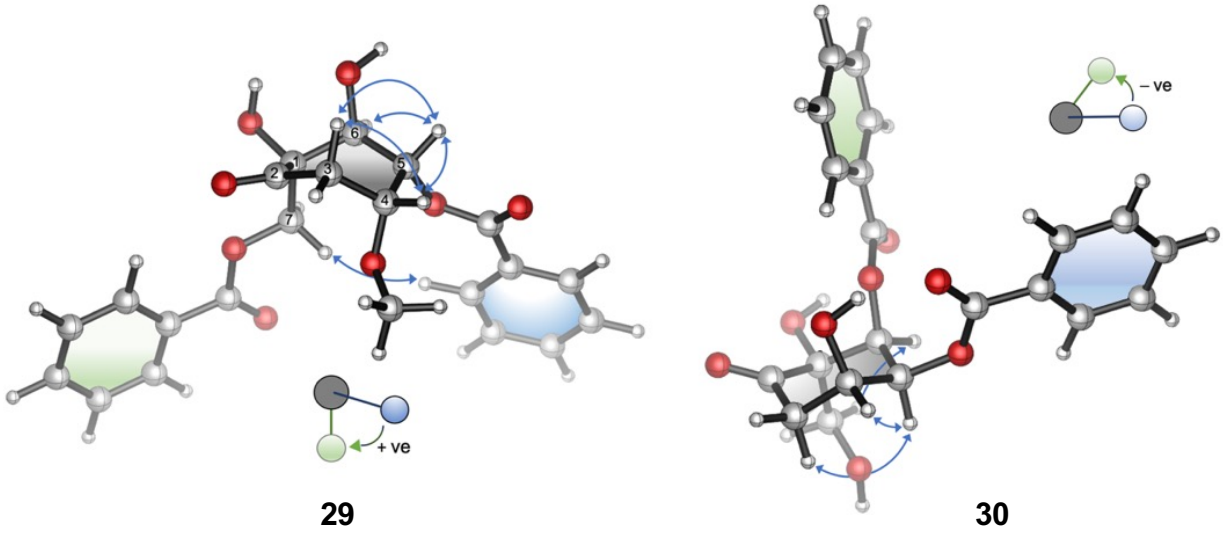


Figure 9. Key NOESY correlations (blue arrows) in **29** and **30** and sign of exciton chirality.

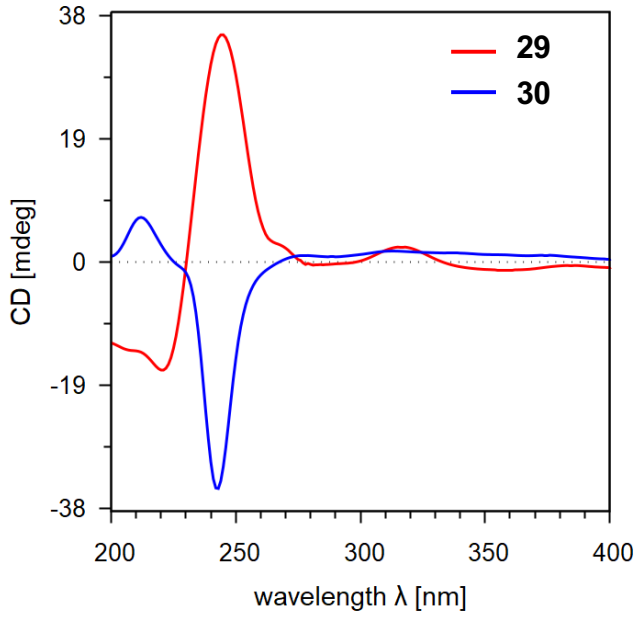


Figure 10. Experimental ECD spectra for compounds **29** and **30**.

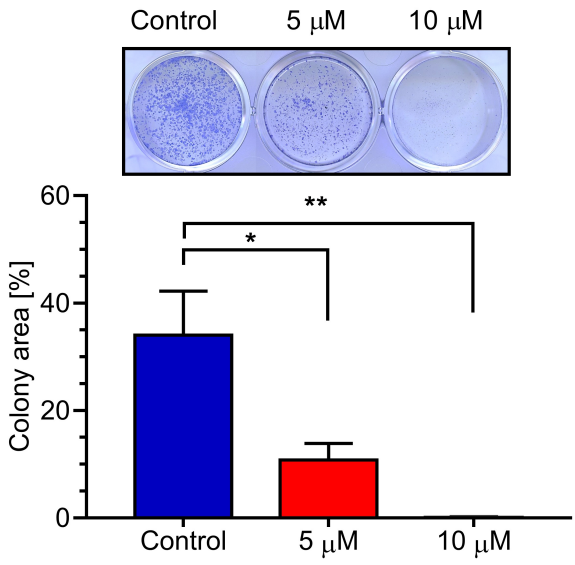


Figure 11. Effect of **29** on PANC-1 cells colony formation.

Chart 5. Structures of compounds isolated from *B. pandurata*

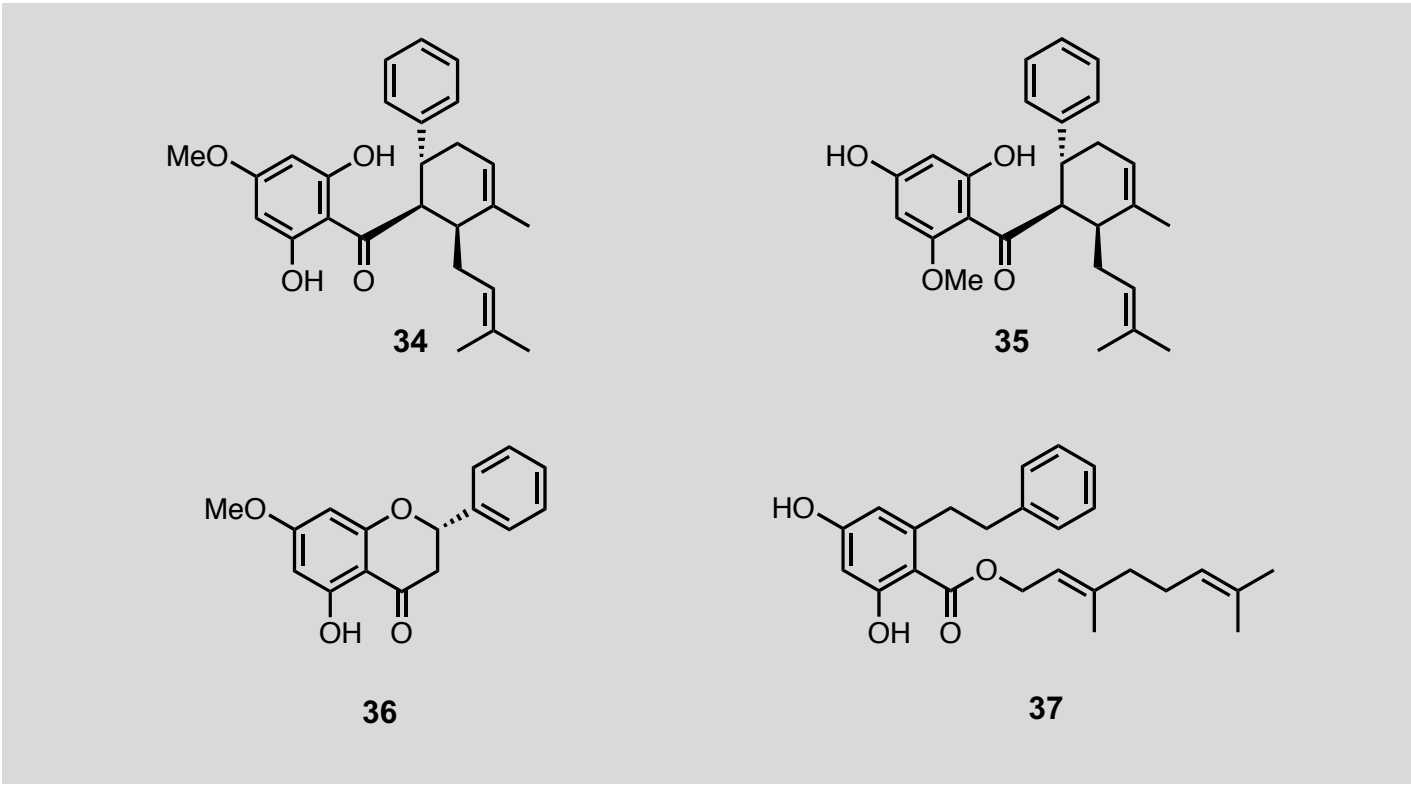


Table 1. Anti-austerity activity (PC_{50}) [b] of the compounds against two human pancreatic cancer cell lines.

Cell lines	**34**	**35**	**36**	**37**	Arct.[a]
PANC-1	1.6	0.9	73	10	0.7
MIA PaCa-2	0.3	0.2	>100	4.8	1.9

[a] Positive control, Arctigenin.

[b] [PC_{50}]: Concentration at which 50% cells were killed preferentially under nutrient nutrient-deprived condition (NDM) but non-cytotoxic under ordinary nutrient rich condition (DMEM) at 24 h.

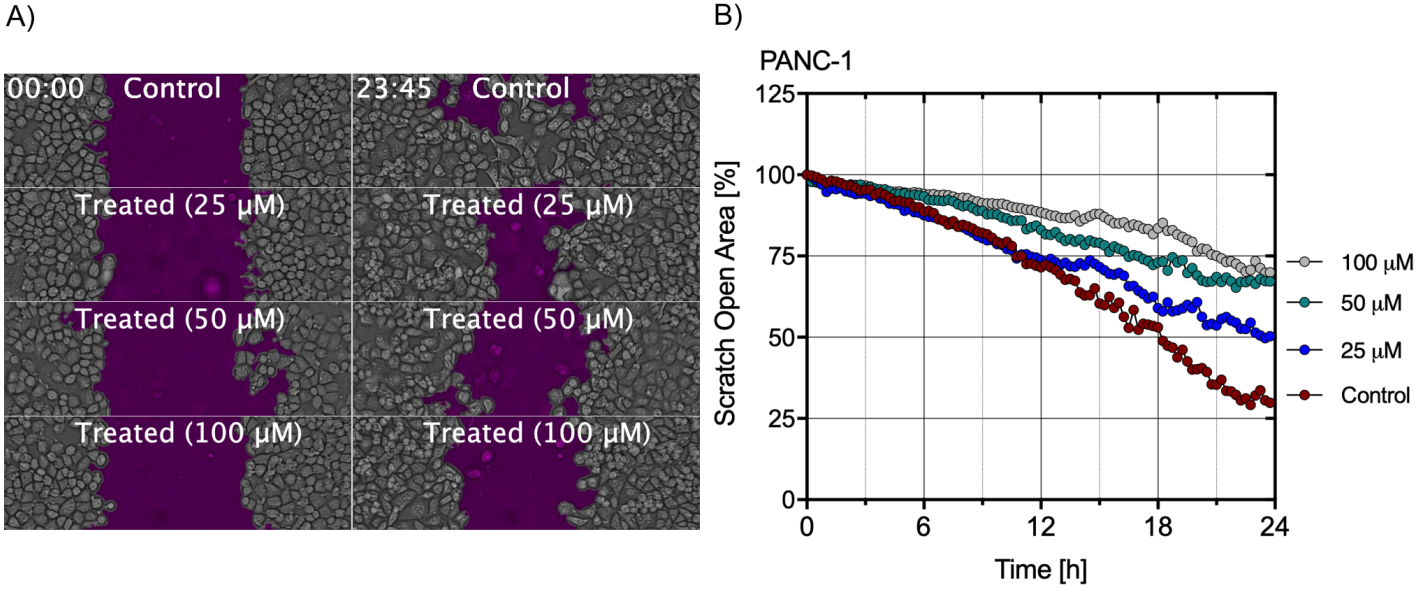


Figure 12. Compound **37** suppresses the migration of PANC-1 in a wound-healing assay in real-time.

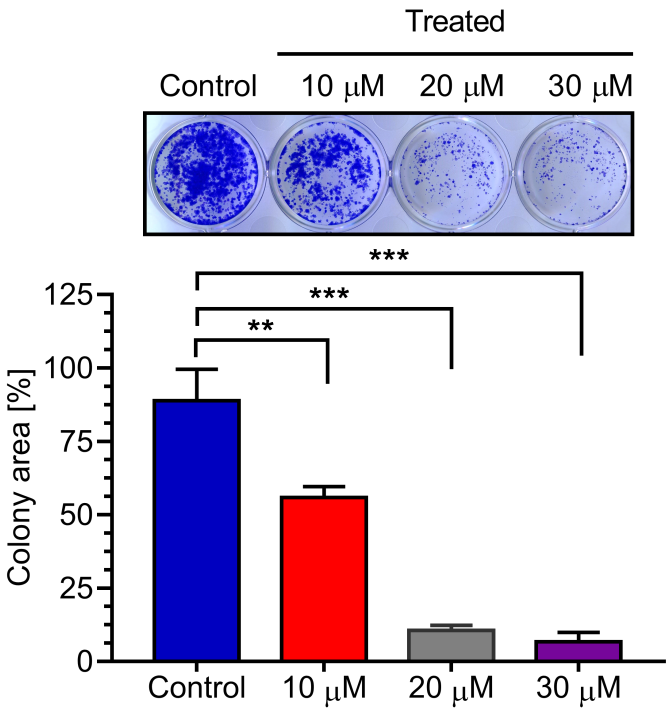


Figure 13. Effect of **34** on PANC-1 cells colony formation.

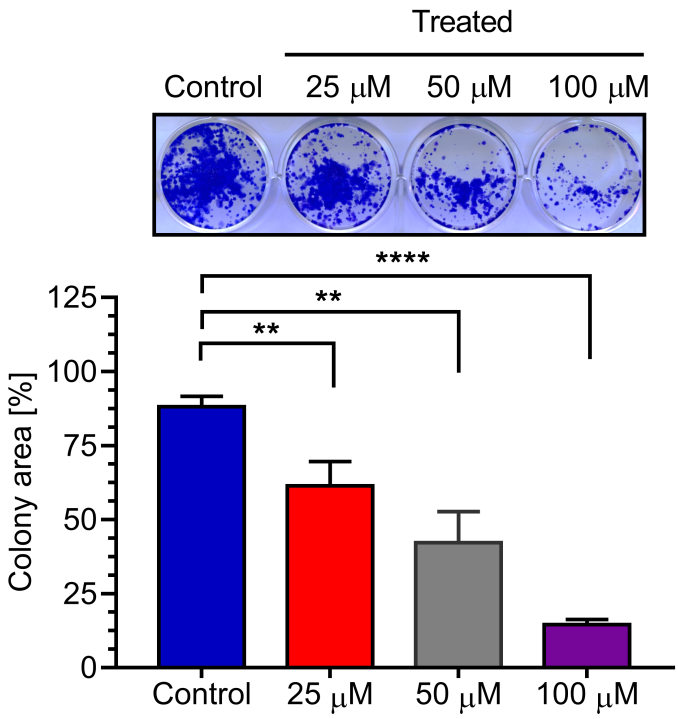


Figure 14. Effect of **37** on PANC-1 cells colony formation.

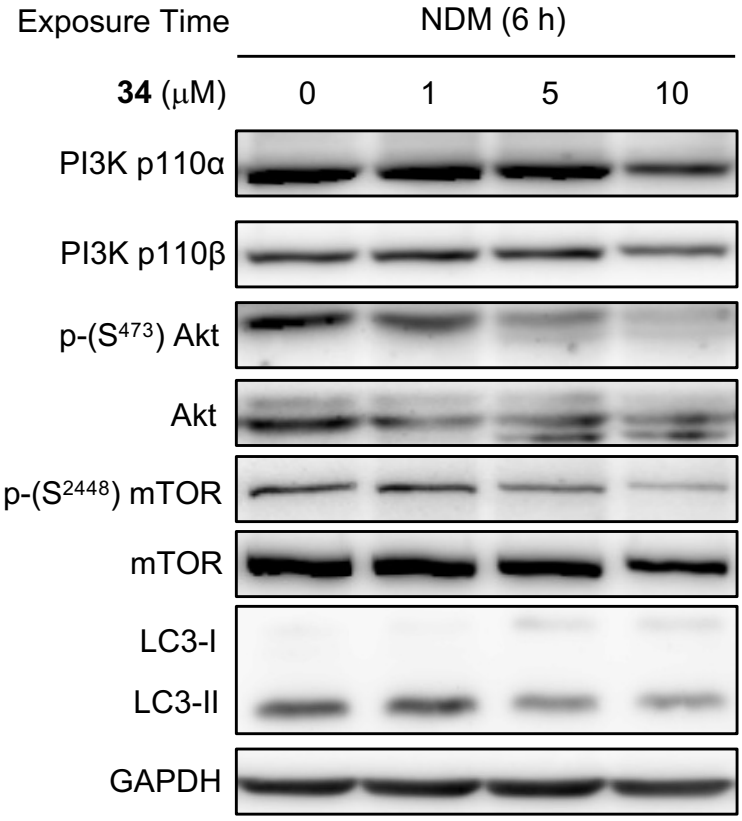


Figure 15. Effect of **34** on the key proteins involved in PI3K/Akt/mTOR/autophagy signaling in PANC-1 cells.

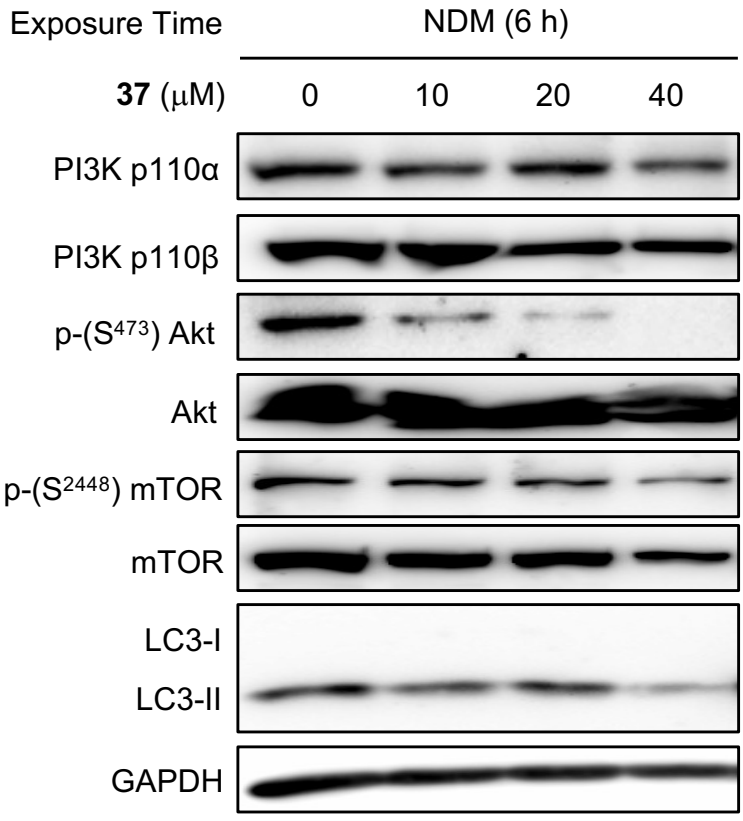


Figure 16. Effect of **37** on the key proteins involved in PI3K/Akt/mTOR/autophagy signaling in PANC-1 cells.