Cytotoxic and antiproliferative activity of thiazole derivatives of Ochraceolide A

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A series of 15 novel 1,3-thiazole amide derivatives of the pentacyclic triterpene Ochraceolide A (1) was synthesized, characterized, and evaluated in vitro against three human cancer cell lines (MCF-7, MDA-MB-231 and SiHa) and a normal cell line (Vero). Synthetic derivatives were obtained by acylation of the 2-aminothiazole triterpene 2, previously reported. Remarkably, the 5-nitrofuramide derivative (2o) showed better cytotoxic and antiproliferative activity than compound 2 and the other derivatives against the three cancer cell lines with CC50 and IC50 values of 1.6–12.7 μM. Furthermore, butyramid derivative (2c) was approximately 25 times more selective than 2, as well as 3.4 times more selective than Docetaxel, against SiHa cells in the cytotoxic assay, while the phenyl amide derivatives were inactive against the three cancer cell lines.
1. Introduction

In 2018, cancer caused 9.6 million deaths, while 18.1 million new cases were estimated worldwide. In women, breast and cervical cancer were the first and third most frequent causes of cancer death, respectively (Ferlay et al. 2019). Despite the advances in cancer chemotherapy, drug resistance provokes treatment failure in approximately 90% of patients, which has led to the continuous searching for new effective drugs (Longley and Johnston 2005).

Natural products have always been an important source of compounds for cancer treatment; then, it is no rare that 33% of small molecules approved between 1981 and 2019, as anticancer drugs correspond to this class of compounds and their synthetic derivatives (Newman and Cragg 2020). Thus, several triterpenoids have been structurally modified to improve their anticancer properties (Laszczyk 2009; Salvador et al. 2017).

A convenient strategy to improve those properties is the fusion of heterocyclic rings to pentacyclic triterpenes scaffolds (Kvasnica et al. 2015; Khwaza et al. 2021), given that 2-aminothiazole derivatives of oleanonic, betulonic and ursolic acid have shown higher cytotoxic activity than their parents (Urban et al. 2012; Borkova et al. 2017, 2020). Hence, we synthesized thiazo Ochraceolide A (2) through the fusion of an aminothiazole ring to the backbone of Ochraceolide A (1) (Herrera-España et al. 2020), a triterpenoid isolated from Elaeodendron trichotomum (Herrera-España et al. 2017). As was expected, its activity against human cervix (SiHa) and breast (MCF-7 and MDA-MB-231) cancer cell lines was more significant than its parent. In this study, as a part of our program to modulate and improve the anticancer potential of modified triterpenoids, we have synthesized 15 acyl amides of thiazo Ochraceolide A and screened for their in vitro cytotoxic and antiproliferative activities against cancerous cell lines MCF-7, MDA-MB-231, and SiHa as well as Vero cells.

2. Results and discussion

The new analogues of 2 include alkyl (2a-2f), phenyl (2g-2l) and furan groups (2m-2o) on the amide moiety (Figure S1, supplementary material). Phenyl and furan groups bear electron-withdrawing and -donating substituents in -para and 5 positions, respectively. For the synthesis of the amide derivatives, aminothiazole 2 was reacted with the corresponding acyl chloride in dichloromethane. The amide derivatives were characterized by $^1$H, $^{13}$C and 2D NMR spectroscopy, HRMS spectrometry (Figures S2–S54, supplementary material), and FT-IR spectroscopy. In the $^1$H and $^{13}$C NMR spectra of the derivatives, the characteristic signals of the triterpene skeleton were observed, and only small changes in the chemical shift were perceived in comparison with those observed in the spectra of 2 (Herrera-España et al. 2020). In the $^1$H NMR spectra, the $\alpha$ protons of compounds 2a-2f were observed at 2.26–2.58 ppm, whereas the typical signals of aromatic protons for 1,4-disubstituted benzene and 2,5-disubstituted furan rings were observed at downfield for derivatives 2g-2o. In $^{13}$C NMR spectra, the signal assigned to the carbonyl group of the $\alpha$-methylene $\gamma$-lactone was observed at approximately 171.5 ppm, and the signal corresponding to the amide group in the 155–174 ppm range. The FT-IR spectra of the derivatives showed two
characteristic bands at approximately 1750 cm$^{-1}$ and 1670 cm$^{-1}$ corresponding to the lactone and amide groups, respectively. The identity of the new derivatives was also confirmed by HR-MS.

According to the literature, in in vitro cellular assays performed with small molecules, relevant activity relates to concentration values below 50 μM (Keseru and Makara 2006). In the present study, the cut-off value adopted to consider a compound as active was an IC$_{50}$ or a CC$_{50}$ < 50 μM. Thus, cervix squamous carcinoma cells (SiHa cells) were sensitive to eight of 15 synthesized amide derivatives. Three of those active compounds (2c, 2d, and 2o) happened to be active against hormone-dependent breast cancer cells (MCF-7 cells). Only one compound (2o) showed activity against MDA-MB-231 (highly invasive triple-negative breast cancer cells), MCF-7, and SiHa cells (Tables S1 and S2, Figures S55–S59, supplementary material).

The CC$_{50}$’s and IC$_{50}$’s showed by 5-nitrofuramide derivative (2o) ranged from 12.7 to 1.6 μM. Indeed, it exhibited the highest cytotoxic (1.6 μM) and antiproliferative (2.9 μM) activities against SiHa cells, improving significantly the anticancer properties of its parent (2). This improvement pattern was also observed against both breast cancer cells but was less pronounced on MDA-MB-231 cells. Furthermore, its selectivity for SiHa cells over normal cells in the cytotoxic assay was ten-fold than that showed by 2, and remarkably, 1.4-fold than Docetaxel (positive control) (Tables S1 and S2, supplementary material).

On the other hand, the 4-nitrobenzamide derivative (2l) exhibited CC$_{50}$’s and IC$_{50}$’s > 50 μM against cancerous and normal cells, hence it was considered a non-active compound (Tables S1 and S2, supplementary material).

The therapeutic action of nitroaromatic compounds (ArNO$_2$) has been related to single- or two-electron bioreduction of their nitro group (Cénas et al. 2021). Indeed, nitrofuran derivatives can elevate the oxidative stress in cells due to the metabolic monoelectronic reduction of their nitro group leads to high reactive oxygen species (ROS) levels (Amaro et al. 2009; Gallardo-Garrido et al. 2020).

The first step of the reduction mechanism involves forming the ArNO$_2$/ArNO$_2^-$ couple, and the energetics of this first electron transfer is described by a midpoint redox potential at pH 7.0 ($E^\prime_{1/2}$). Notably, the $E^\prime_{1/2}$’s of the most important groups of ArNO$_2$ decrease in the following order: nitropyridines > nitrofurans ≥ nitrothiophenes > nitrobenzenes > nitroimidazoles; thus, nitrofurans derivatives have a higher tendency to be reduced than nitrobenzene derivatives (Cénas et al. 2021).

Therefore, the remarkable cytotoxic activity of 2o on SiHa cells can be attributed to the presence of nitrofuran moiety, and the significant decreasing of anticancer activities of 2l correlates reasonably well with the difference between midpoints redox potential of 2-nitrofuran ($E^\prime_{1/2} = -0.330$) and nitrobenzene ($E^\prime_{1/2} = -0.485$) groups (Cénas et al. 2021).

The butyramide derivative (2c) showed anticancer properties toward SiHa cells (CC$_{50} = 8.6$, IC$_{50} = 9.5$ μM) approximately four-fold less than to those shown by compound 2o; therefore, it was considered the second most active compound against this class of cancerous cells (Tables S1 and S2, supplementary material). Remarkably, the selectivity of its cytotoxic activity (SI = 20.2) was the highest found, being 25 and 3.4 times higher than that shown by its precursor (2) and the positive control,
respectively. Moreover, 2c resulted 5.6-fold more selective than valeramide (2e), 2.3-fold than isobutyramide (2d) and 2.8-fold than isovaleramide (2f), while the propionamide derivative (2b) was considered non-active. These facts seem to reveal that the selectivity of the synthesized derivatives has a slight relationship with the length of their hydrocarbon chains (Table S1, supplementary material).

3. Experimental

See supplementary material.

4. Conclusion

The obtained results suggest that acylation of the amino group of compound 2 affects its anticancer activity and that it is plausible to improve the antiproliferative or cytotoxic activities, as well as selectivity of 5-nitrofuramide of thiazo Ochraceolide A through the alkylation of the furan ring. However, further studies on the mechanism of action of these derivatives are required to understand the structure-activity relationship better.

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Disclosure statement

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