

Bortezomib potentiates the antitumor effect of tributyltin(IV) ferulate in colon cancer cells exacerbating ER stress and promoting apoptosis.

Adriana Celesia^{1#}, Tiziana Fiore^{2#}, Diana Di Liberto¹, Michela Giuliano^{3*}, Claudia Pellerito^{2§*}, and Sonia Emanuele^{1§}.

¹ Dipartimento di Biomedicina, Neuroscienze e Diagnostica avanzata (BIND), Università degli Studi di Palermo, Via del Vespro 129, 90127 Palermo, Italy. adriana.celesia@unipa.it, diana.diliberto@unipa.it, sonia.emanuele@unipa.it.

² Dipartimento di Fisica Chimica - Emilio Segrè (DiFC), Università degli Studi di Palermo, Viale delle Scienze, Ed. 17, 90128 Palermo, Italy - CIRCMSB – Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici, Via Celso Ulpiani, 27, 70125 Bari, Italy. tiziana.fiore@unipa.it, claudia.pellerito@unipa.it.

³ Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Plesso di Biochimica, Via del Vespro 129, 90127 Palermo, Italy. michela.giuliano@unipa.it.

These Authors equally contributed to this paper as first Author.

§ The Authors share senior co-authorship.

* Correspondence: claudia.pellerito@unipa.it; michela.giuliano@unipa.it,

Abstract

Organotin(IV) complexes represent promising drugs in medicinal chemistry for their potential use in cancer therapy. We recently reported synthesis and characterization of a new organotin(IV) complex of ferulic acid (FA), tributyltin(IV) ferulate (TBT-F), showing its antitumor action in colon cancer cells. Here we provide evidence that the efficacy of this compound is strongly potentiated by the proteasome inhibitor bortezomib (BTZ).

While low concentrations of tributyltin(IV) ferulate alone promoted autophagy without reducing cell viability, combination of the two compounds markedly affected colon cancer cell viability, cell morphology and exasperated endoplasmic reticulum (ER) stress, as revealed by a dramatic increase in Grp78 ER stress marker and consequent unfolded protein response (UPR) pathway components including PERK, eIF2 α and downstream transcription factor Chop. Our data also indicated that ER stress promoted by the TBT-F/BTZ combination leads to apoptosis, as shown by caspase 3 activation, increase in p53 levels, chromatin condensation and prevention of the TBT-F effect by the caspase inhibitor zVAD-fmk.

Keywords: Organotin(IV) complexes, ferulic acid, proteasome inhibitor, ER Stress, apoptosis

1. Introduction

Many organotin compounds have been considered as potential cancer chemotherapeutic agents because of their apoptotic inducing properties [1–5], and nowadays they occupy an important place in cancer chemotherapy reports [6–8]. Enhancement or modulation of organotin(IV) moieties efficacy can be achieved by combination with ligands possessing biological activity or acting as carrier for specific tissue districts, thus representing new potential targeting therapy. Among these ligands, ferulic acid (FA) represents a good candidate due to its biological properties. FA is a natural compound that has been widely studied for its antioxidant action and therapeutic effects [9,10]. Recent findings also support a possible role of FA in cancer therapy, partially based on its antioxidant potential. FA has been shown to suppress reactive oxygen species (ROS) that often result increased in tumor cells unbalancing the redox equilibrium towards an oxidative condition that favours tumor growth and drug resistance [11]. However, due to beneficial antioxidant effects to the cells, the antiproliferative action of FA is usually exerted at quite high concentrations [10,12].

In order to improve the antitumor efficacy of FA, we produced the chemical derivative tributyltin(IV) ferulate (TBT-F) that couples ferulate with an organotin moiety [13]. We previously demonstrated that this compound, rather than an antioxidant, behaves as a pro-oxidant and induces endoplasmic reticulum (ER) stress and autophagic cell death in different colon cancer cell lines [14]. Autophagy and oxidative stress are strictly related and represent key processes in cancer therapy [15,16]. Autophagic cell death becomes particularly important to target tumor cells that display resistance to classic apoptosis and can, thus, represent a nice tool to limit tumor progression and improve the efficacy of anti-cancer treatments [17]. In our conditions, we could not detect apoptosis following colon cancer treatment with TBT-F [13].

Therefore, we further considered the possibility to combine TBT-F with pro-apoptotic drugs that can potentiate its antitumor effects acting with a different mechanism. In this perspective, we chose the proteasome inhibitor bortezomib, since it represents a widely documented apoptotic inducer in tumor

cells [18,19] and also a strong promoter of the unfolded protein response (UPR) due to proteasome inhibition [20,21].

Targeting the ubiquitin-proteasome degradation system has represented an attractive tool to specifically attack tumor cells [22]. However, while different proteasome inhibitors have been approved in the therapy of hematological malignancies including multiple myeloma, the clinical outcome of treatment with these drugs in solid tumors was unexpectedly lower [23]. As suggested by Zagirova et al. [24], this is most likely due to the heterogeneity of proteasome complexes in solid tumors including colon cancer. Moreover, evidence has been provided that nuclear export of ubiquitinated proteins can modulate colon cancer cell sensitivity to proteasome inhibition [25]. Interestingly, several studies propose combinations of bortezomib with other anti-tumor compounds [26,27] or radiotherapy [28] in order to overcome resistance mechanisms and improve colon cell sensitivity to proteasome inhibition.

Considering that no data are present in the literature regarding combination of bortezomib with organotin compounds, the aim of the present paper is to provide a rationale to combine bortezomib with the novel compound TBT-F and to propose a mechanism that accounts for their potentiation in colon cancer cells.

2. Materials and methods

Chemicals and reagents for the synthesis and physical measurements of tributyltin(IV) ferulate (TBT-F) and computational methods were already described in our previous paper [13].

2.1 Cell cultures

Human colon cancer HCT116 and Caco2 cells were cultured in RPMI 1640 medium containing 10% (v/v) fetal bovine serum (FBS), 2.0 mM glutamine and antibiotic, antimycotic solution (100 U/mL penicillin, 100 µg/mL streptomycin) in a humidified atmosphere at 37 °C in the presence of 5% CO₂. For the experiments, cells were seeded in 96 well-plates (7x10³) or 6 well-plates (15x10⁴). Cells were allowed to adhere overnight and subsequently treated with the compounds or vehicle alone. For the

experiments, TBT-F and BTZ were dissolved in DMSO and prepared as 10 mM stock solutions TBT-F and 5 mM stock solution BTZ, which were opportunely diluted to final concentrations in culture medium before use. The final concentration of DMSO in the medium did not exceed 0.05% (v/v), which did not affect cell viability as confirmed by a control consisting in equal volumes of DMSO that were added to untreated cells.

2.2 MTT assay cell viability evaluation

The effect of the compounds on cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described. Values reported in the figures are expressed as percentage of the viability of untreated cells and are the means $\pm$ SD of four independent experiments.

2.3 Autophagy determination by monodansylcadaverine (MDC) labeling

Autophagy induction was evidenced by MDC staining of autophagic vacuoles that was performed as previously described [14]. Cells were treated in 96-well plates with the compounds for the established times, then stained with 0.05 mM MDC in culture medium at 37 °C for 10 min. Following incubation, cells were washed with PBS and immediately analysed under a Leica DMR fluorescence microscope (Wetzlar, Germany) using an appropriate 4',6-diamidino-2-phenylindole (DAPI) filter (excitation wavelength of 372 nm and an emission wavelength of 456 nm). Images were photographed and captured by a computer-imaging system (Leica DC300F camera and Adobe Photoshop for image analysis).

2.4 Nuclei morphology evaluation by Hoechst 33342 staining

In order to evaluate nuclear morphology and integrity, cells were stained with Hoechst 33342, as previously described [29]. Briefly, cells (8×10^3) were plated in a 96-well plate treated with the compounds for the established times and stained with Hoechst 33342 (2.5 μ g/mL) for 30 min. Following washings with PBS, cells were visualized using an inverted Leica fluorescent microscope (Leica Microsystems, Wetzlar, Germany) endowed with a 40,6-diamidino-2-phenylindole dihydrochloride (DAPI) filter. Leica Q Fluoro software was used for image acquisition.

2.4 Western blotting analysis

Protein extracts were prepared by incubating cell pellets for 20 min in ice-cold RIPA lysis buffer supplemented with a cocktail of protease inhibitors and sonicated for three times for 10 s, as previously reported [30]. Proteins were quantified by Bradford assay and equal amount of proteins (30 μ g) was separated by SDS-PAGE and then electrotransferred to a nitrocellulose membrane for immunodetection. The blots were developed using electrochemical luminescence labeling systems by ChemiDoc, XRS (Bio-Rad Laboratories, Hercules, CA, USA) Image system. Optical densities of the bands were analysed with Quantity One Imaging Software (Bio-Rad Laboratories). The correct protein loading was verified by means of both Ponceau red staining and immunoblotting for the housekeeping protein γ -tubulin. The results showed in the figures are representative of three independent experiments with similar results. The results of densitometric analysis are reported as the ratio of the intensity of the bands of treated samples to untreated ones, after normalization with γ -tubulin.

2.5 Statistical analysis

Data were analysed using Student's *t*-test using GraphPadPrism™ 4.0 software (graphPadPrism™ Software Inc., San Diego, CA, USA) and reported as means $\pm$ S.D. Comparisons between the control (untreated) vs. treated samples were made. If a significant difference was detected by ANOVA analyses, this was re-evaluated by post-hoc Bonferroni's test. GraphPadPrism™ 4.0 Software (Graph PadPrism™ Software Inc., San Diego, CA, USA) was used for statistical calculations. The statistical significance threshold was fixed at $p < 0.05$.

3. Results and discussion

3.1 Chemical synthesis and structural characterization

The research on the synthesis and spectroscopic characterization of a new complex of ferulic acid has resulted in a promising new compound with general formula $\text{Bu}_3\text{Sn}(\text{L})$, as we previously reported in [13]. The analytical data, and the IR spectrum of tributyltin(IV) ferulate complex suggest that the tin

atom in such derivative is at least five coordinated, with the ligand acting as a monoanionic bidentate ligand, through a bridging carboxylate anion. In the free ligand, the C=O stretching of the carboxylic group was observed at 1692 and 1666 cm^{-1} . These bands disappeared upon coordination, indicating deprotonation of the carboxylic group. At the same time, two new bands were observed at 1565 cm^{-1} and 1392 cm^{-1} , assigned respectively to asymmetric and symmetric COO^- stretching modes. As previously reported, $\Delta\nu$ values [$\Delta\nu = \nu_{\text{as}}(\text{COO}^-) - \nu_{\text{s}}(\text{COO}^-)$] are indicative of the coordinating mode of the carboxylate [31]. In our case, $\Delta\nu$ value (173 cm^{-1}) is characteristic for coordinated bridging carboxylate groups. Finally, absorption bands characteristic of $\nu(\text{Sn-C})$ and $\nu(\text{Sn-O})$ present at 577 cm^{-1} and at 453 cm^{-1} respectively, support the complexation of tin(IV) with the ligand (Fig. 1) [32,33].

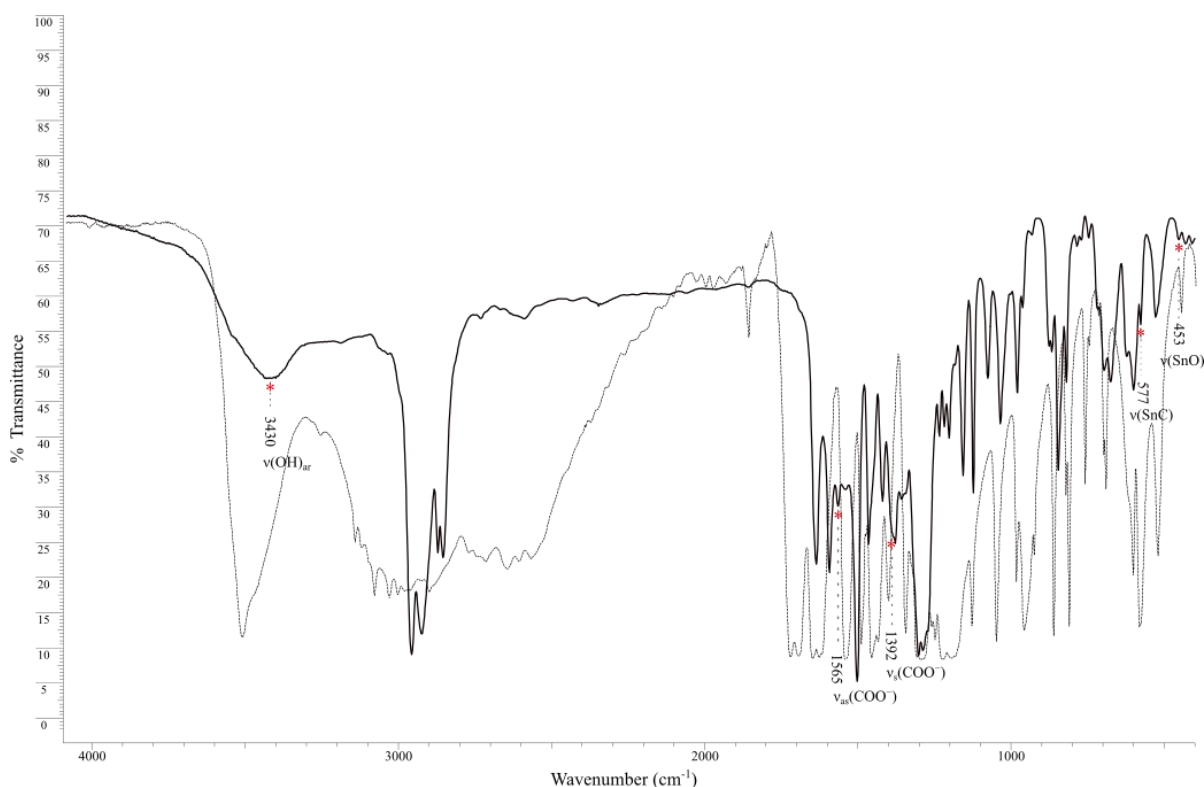


Fig. 1. Overlapped FT-IR spectra for FA (dashed line) and TBT-F (full line).

ESI-MS spectra of **TBT-F** show typical feature of the organotin complexes spectra. The assignments of the individual ions are based on the combination of positive-ion and negative-ion mass

spectrometric experiments, and supported by comparison between theoretical and experimental isotopic distributions of monomeric (Fig. 2) and dimeric (Fig. 3) ions.

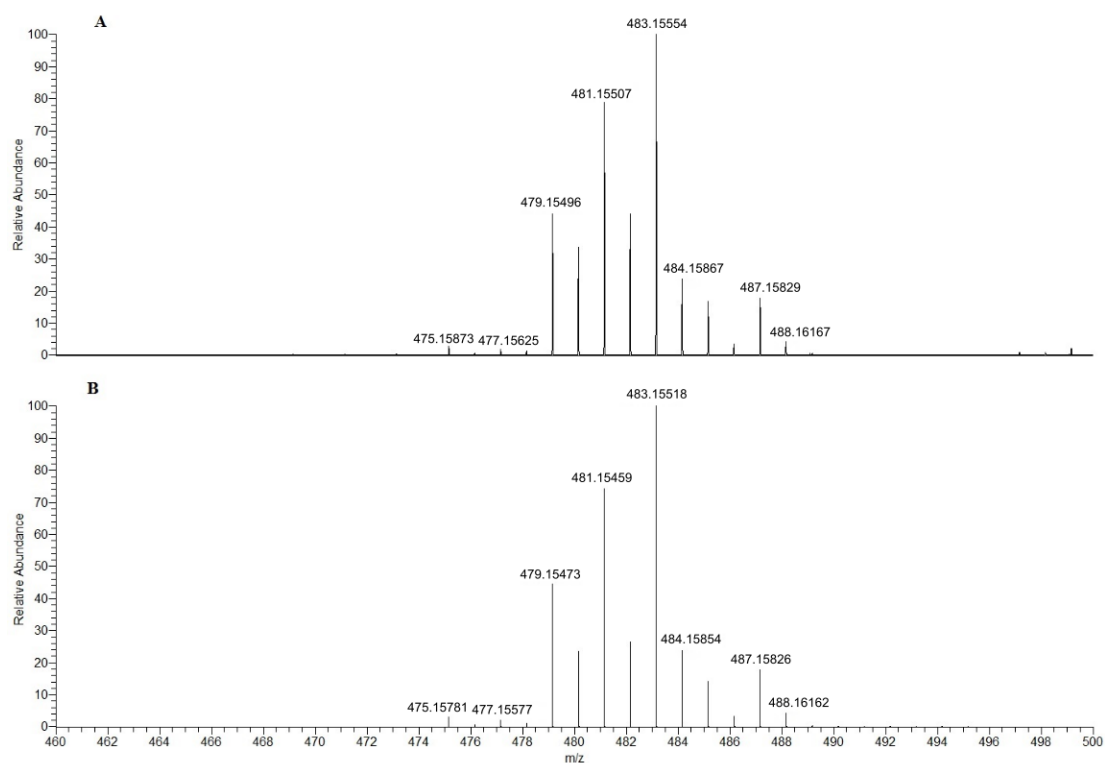


Fig. 2. Experimental (A) and theoretical (B) isotopic distributions of monomeric ion, $[\text{TBTF-H}]^-$.

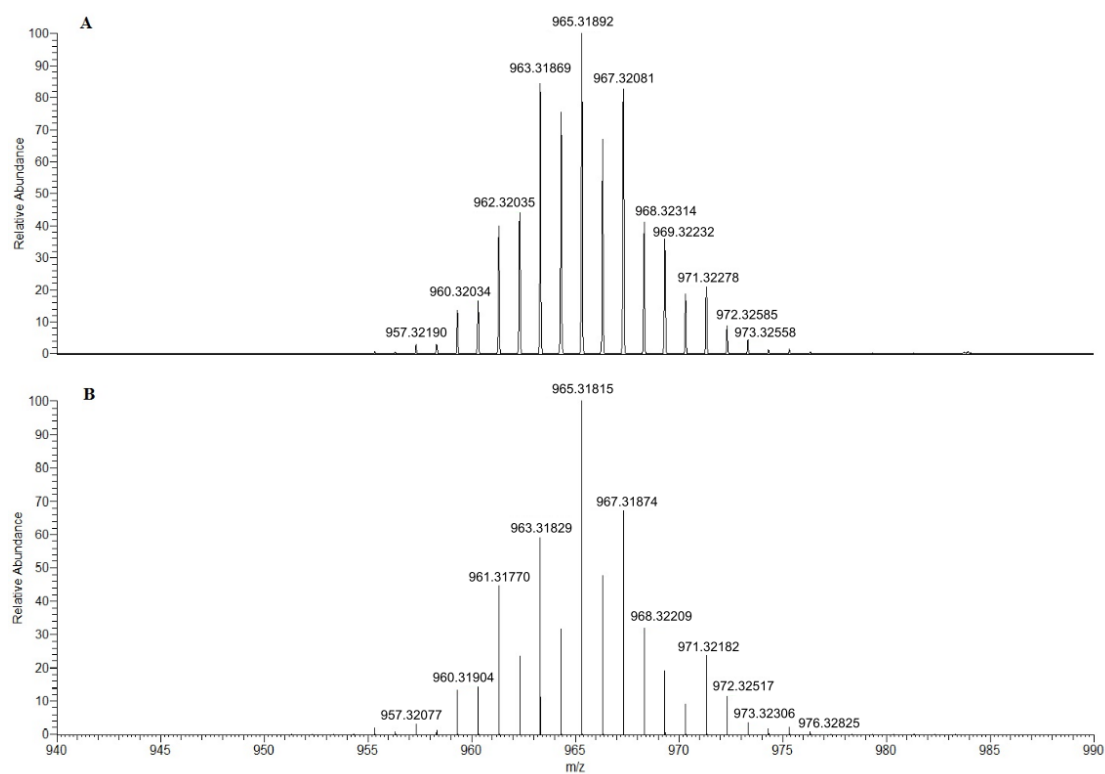


Fig. 3. Experimental (A) and theoretical (B) isotopic distributions of dimeric ion, $[2\text{TBTF-H}]^-$.

In addition, spectroscopic UV–vis data show that the formation of metal complex causes changes in the electronic charge distribution within the molecule compared with acid (Fig. 4).

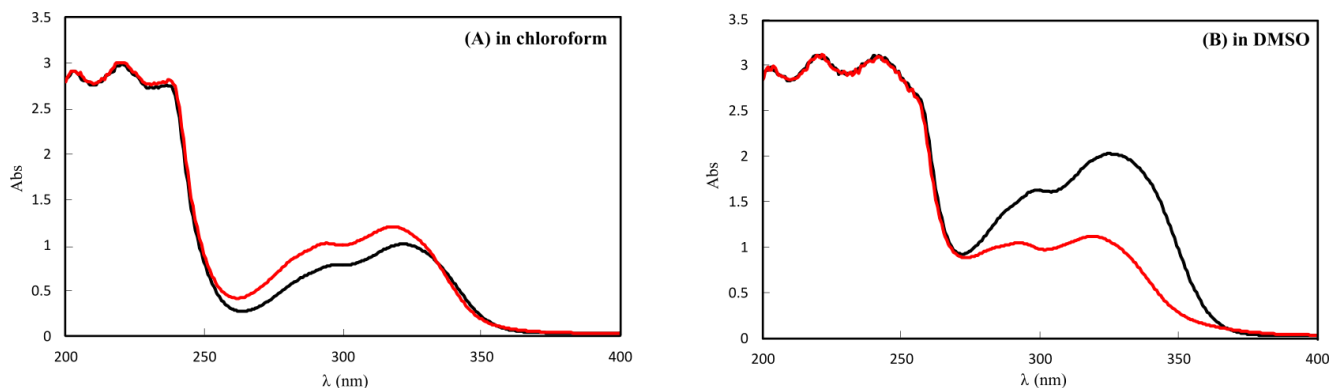


Fig. 4. Overlapped UV–vis spectra of chloroform (A) and DMSO (B) solutions containing a constant concentration of FA (black) and TBT-F (red) (1×10^{-4} M).

NMR investigation in CDCl_3 solution indicated that the bidentate nature of the ligand is lost in solution and that tin has a four-coordinated geometry. Differently, in DMSO-d_6 solution tin is penta-coordinated and this coordination is attained more likely considering the coordination of a solvent molecule on the tin coordination sphere.

DFT calculations support the interpretation of these experimental evidences.

Experimental data and tables were reported in reference [13].

3.2 Biological study

Our previous papers demonstrated that TBT-F is very effective as antitumor agent in colon cancer cells promoting ER stress and type 2 (autophagic) programmed cell death [14]. Based on these evidences, we decided to perform combination studies in order to reduce the TBT-F concentrations and to investigate new biochemical action mechanisms. Specifically, we considered the proteasome inhibitor bortezomib (BTZ) as a possible candidate that can improve TBT-F efficacy since it can increase ER stress and consequent cell death due to proteasome inhibition.

3.2.1 The effect of TBT-F/BTZ combination on colon cancer cell viability

According to our previous results [13], for cell viability experiments, 200 nM TBT-F was chosen as a reference point being the concentration that induces about 50% reduction in cell viability in colon cancer cells. In addition, 100 nM was considered as a TBT-F subtoxic concentration. For combination experiments, the effects of two subtoxic concentrations of BTZ (5 and 10 nM) were evaluated in association with 100 and 200 nM TBT-F in colon cancer HCT116 and Caco-2 cells by MTT assay. Interestingly, BTZ markedly increased the cytotoxic effect of TBT-F in both cell lines in a dose dependent manner (Fig. 5). In particular, after 48 h treatment the maximum effect was obtained with the combination 200 nM TBT-F/10 nM BTZ (-82% in HCT116 and -75% in Caco-2 cells) compared to single treatments. Considering the higher efficacy of the combination 100 nM TBT-F/ 10 nM BTZ in HCT116 cells, these conditions were considered for the further evaluations.

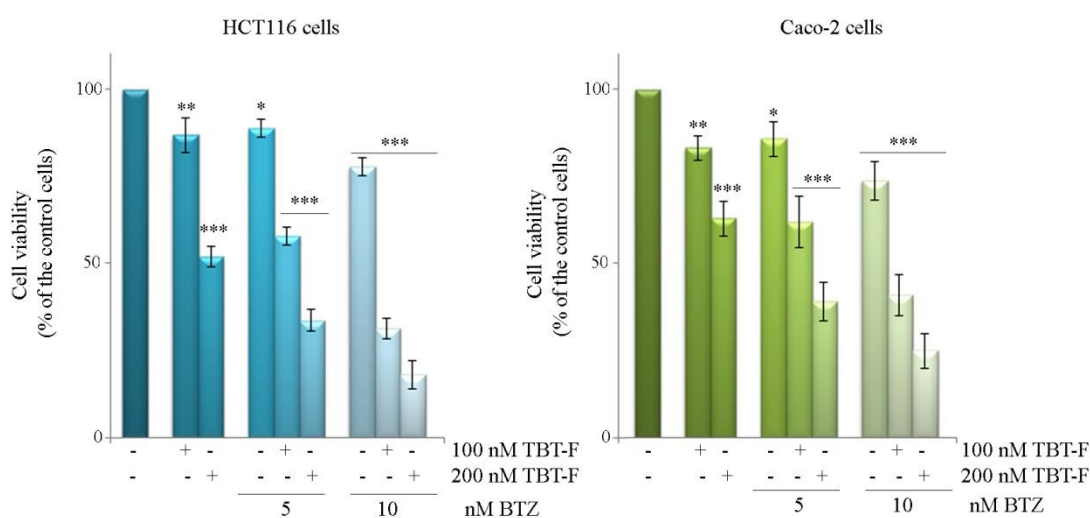


Fig. 5. Cytotoxic effects exerted by TBT-F/BTZ combination in colon cancer cells. Colon cancer cell viability was measured by MTT assay, as reported in Methods. HCT116 and Caco-2 cells were incubated for 48 h in the presence of the indicated doses of tributyltin(IV) ferulate (TBT-F) or Bortezomib (BTZ) employed alone or in combination. The results reported in the histograms are the means of four separate experiments $\pm$ SD. (*) p -value < 0.05, (**) p -value < 0.01; (***) p -value < 0.001 compared with untreated cells.

3.2.2 The effect of TBT-F/BTZ combination on autophagic and apoptotic pathways

Cell viability results were confirmed in HCT116 cells by evaluation of cell morphology. In particular, Fig. 6A shows that treatment for 48 h with subtoxic concentrations of TBT-F or BTZ only produced a reduction in cell number, most likely due to a cytostatic effect, but the cells clearly appeared viable. In contrast, TBT-F/BTZ combination determined typical morphological features of cell death including round shape morphology and detachment from the substrate.

It is well known that BTZ induces apoptosis in a wide variety of tumor cell lines [20]. On the other hand, we showed that TBT-F is capable of stimulating autophagic cell death in colon cancer cells [13,14]. In order to investigate whether cell viability reduction obtained with the combination TBT-F/BTZ was due to apoptosis or autophagic cell death, we assessed autophagic vacuolization by monodansylcadaverine (MDC) staining and chromatin condensation by Hoechst staining. As shown in Fig. 6B, TBT-F alone induced positivity to MDC staining, consisting in the appearance of green fluorescent dot-like structures in the cells, confirming our previous findings [14]. On the other hand, MDC positivity was not detected neither in cells treated with BTZ alone nor in those treated with the combination TBT-F/BTZ. Although data present in the literature indicate that active doses of BTZ can trigger cytoprotective autophagy, a mechanism that accounts for cell resistance to the drug [34], according to our results, we concluded that autophagy is not activated by such low BTZ concentrations. It is interesting to note that autophagy did not even occur following TBT-F/BTZ combination whereas only in this condition chromatin condensation and fragmentation was observed by Hoechst staining as a clear sign of cell death (Fig. 6C).

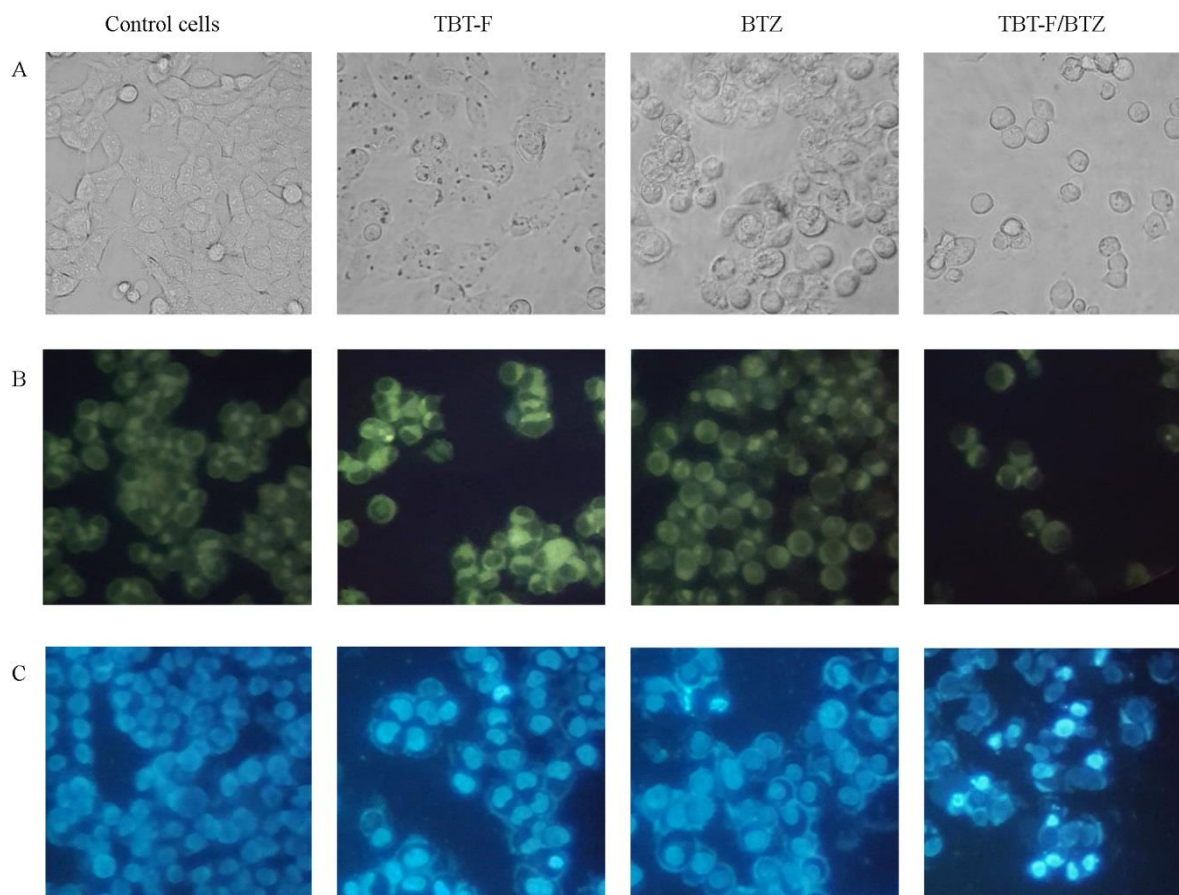


Fig. 6. Microscopic evaluation of TBT-F and BTZ effects. (A). Morphological analysis of HCT116 cells treated for 48 h in the presence of 100 nM TBT-F, 10 nM BTZ employed alone or in combination. Cells were visualized under light microscope at 400x magnification. (B). Intracellular vacuolization induced by treatment for 48 h with the compounds, was evaluated after the addition of 50 μ M monodansylcadaverine (MDC) for 10 min. The visualization was under a fluorescence microscope equipped with a 4',6-diamidino-2-phenylindole (DAPI) filter at a magnification of 400 $\times$. Pictures are representative of three fields from two independent experiments. (C). Nuclear DNA fragmentation observed after treatment for 48 h with the compounds. At the end of incubation, cells were stained with the vital dye Hoechst 333428 and visualised under fluorescence microscope Leika equipped with a DAPI filter (magnification of 400 $\times$).

To confirm the different effect on autophagic and apoptotic pathways induced by TBT-F/BTZ combination, we pre-treated the cells with the autophagy inhibitor Bafilomycin A1 or with the pan-caspase inhibitor zVAD-fmk, respectively. The results reported in Fig. 7 indicate that the effect of the combination was almost completely prevented by z-VAD-fmk while it was not significantly modified by Bafilomycin A1. These results strongly suggest that the combination TBT-F/BTZ activates a caspase-dependent death mechanism.

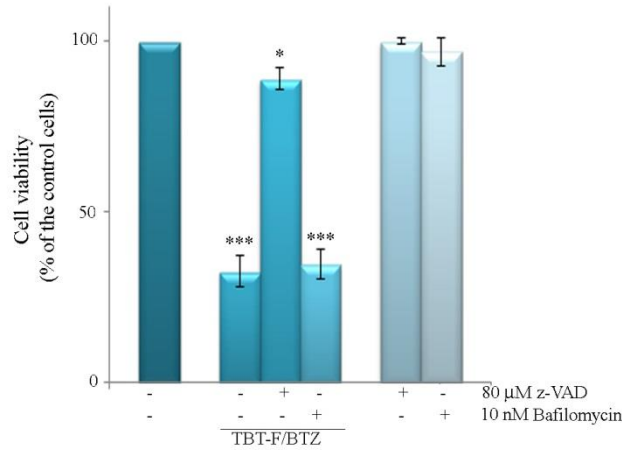


Fig. 7. Effects exerted by the caspase inhibitor zVAD-fmk and autophagy inhibitor Bafilomycin A1 on TBT-F/BTZ-induced cytotoxicity. Cells were pre treated for one hour with 80 μ M zVAD-fmk or with Bafilomycin A1. Then, combination TBT-F/BTZ was added and treatment protracted for additional 48h. Cell viability was determined by MTT assay as described in Methods. The results reported in the histograms are the means of four separate experiments $\pm$ SD. (*) p -value < 0.05, (***) p -value < 0.001 compared with untreated cells.

3.2.3 TBT-F/BTZ combination induces ER stress and apoptosis

Several evidences indicate that BTZ induces ER stress in different types of hematological malignancies [35,36]. Moreover some resistance mechanisms to BTZ have been shown to be somehow related with defects in ER stress [37,38].

Based on these evidences, we asked ourselves whether BTZ could also stimulate ER stress in colon cancer cells and whether ER dysfunction could be responsible for potentiation of TBT-F action. This hypothesis was also sustained by our previous finding that active TBT-F doses strongly promote ER stress in colon cancer cells [14]. We, thus, analysed some ER stress markers after treatment of HCT116 cells with subtoxic concentrations of BTZ and TBT-F either alone or in combination.

Western blot analysis shown in Fig. 8 indicated that BTZ alone was capable of remarkably increasing the levels of Glucose-regulated protein 78 (Grp78), a major ER BiP chaperone involved in protein quality control and in ER-transmembrane signalling [39]. Upon Grp78 sequestration by misfolded proteins, components of the unfolded protein response such as Protein Kinase R-like ER Kinase (PERK) initiate a signal cascade that culminates in activation of transcriptional factors that can direct

cell fate. Among these factors, Chop plays a key role because it is responsible for triggering cell death in case of a prolonged ER-stress condition [40]. Our data indicated that BTZ alone was able to increase the levels of PERK with the consequent phosphorylation of its target eIF2 α (Eukaryotic initiation Factor 2) as well as increase in Chop (CCAAT-enhancer-binding protein homologous protein) levels. Concerning TBT-F alone, this low concentration did not significantly modify the level of Grp78 but slightly increased PERK and significantly increased eIF2 α phosphorylation and Chop levels. It is interesting to note that the combination TBT-F/BTZ markedly potentiated all the increasing effects on the ER stress markers starting from Grp78 and culminating in Chop levels. Another interesting observation was the ability of BTZ alone to induce a dramatic increase in the level of p53, a factor which is strictly related to different cell death pathways, including classic apoptosis [41]. The effect on p53 levels was also observed with the combination of the two compounds but not with TBT-F alone. Moreover, only the combination (and not the single compounds) induced a remarkable decrease in the level of pro-caspase 3 that we interpreted as caspase 3 activation, a finding that is in line with the protection exerted by the pan-caspase inhibitor zVAD-fmk and strongly suggesting the involvement of a classic apoptotic pathway.

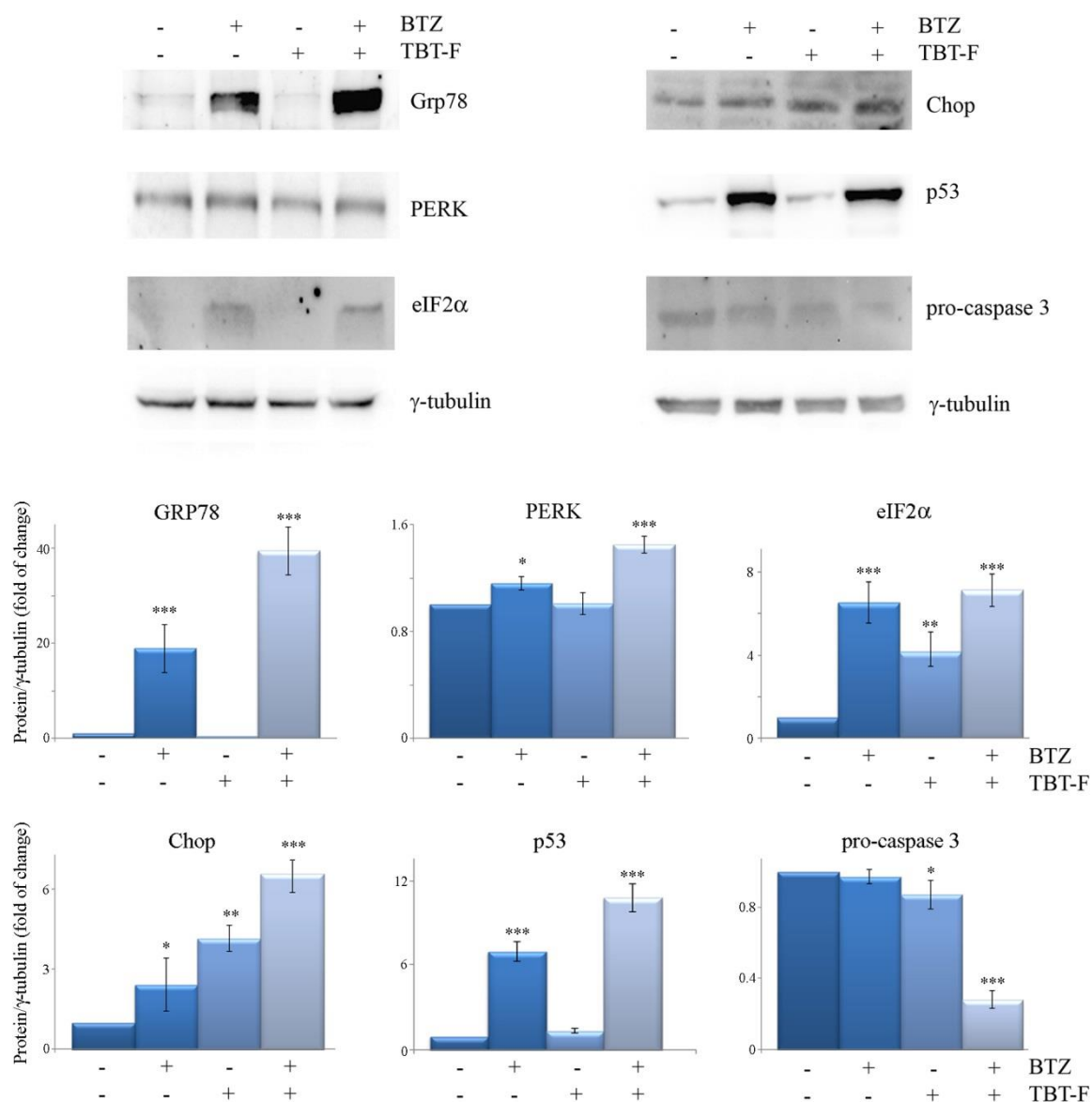


Fig. 8 Combination TBT-F/BTZ induced ER stress and apoptotic markers. Western blot analysis of ER stress markers (left panel) and apoptotic markers (right panel). Cells were incubated for 48 h in the presence of TBT-F and BTZ either alone or in combination. Protein extracts were then prepared as described in Methods and subjected to western blot analysis. The correct protein loading was ascertained evaluating γ -tubulin levels. Representative blots of three independent experiments and densitometric analysis are shown. (*) p -value < 0.05; (**) p -value < 0.01; (***) p -value < 0.001 compared with untreated cells.

4. Conclusion

The results reported indicate that the proteasome inhibitor BTZ is capable of potentiating the anti-tumor effect of TBT-F. Mechanistically, BTZ exacerbated ER stress most likely due to increased

unfolded protein response consequent to proteasome inhibition. Exasperation of ER stress led to Chop and p53-induced apoptosis, events that were not observed with TBT-F alone.

These data represent the first evidence that the two compounds biochemically cooperate and provide a basic rationale for their combination in translational studies.

Acknowledgments

The Authors gratefully acknowledge the financial support of the Università degli Studi di Palermo, Italy (FFR_D08_FIORE, FFR-D15_GIULIANO).

References

- [1] M. Nath, M. Vats, P. Roy, *Eur. J. Med. Chem.* 59 (2013) 310–321. <https://doi.org/10.1016/j.ejmech.2012.11.023>.
- [2] M. Nath, M. Vats, P. Roy, in: M. Gupta Bhowon, S. Jhaumeer-Laulloo, H. Li Kam Wah, P. Ramasami (Eds.), *Chem. Sustain. Dev.*, Springer Netherlands, Dordrecht, 2012: pp. 301–315. https://doi.org/10.1007/978-90-481-8650-1_18.
- [3] S. Tabassum, C. Pettinari, *J. Organomet. Chem.* 691 (2006) 1761–1766. <https://doi.org/10.1016/j.jorganchem.2005.12.033>.
- [4] C. Pellerito, P. D’Agati, T. Fiore, C. Mansueto, V. Mansueto, G. Stocco, L. Nagy, L. Pellerito, *J. Inorg. Biochem.* 99 (2005) 1294–1305. <https://doi.org/10.1016/j.jinorgbio.2005.03.002>.
- [5] H. Stridh, I. Cotgreave, M. Müller, S. Orrenius, D. Gigliotti, *Chem. Res. Toxicol.* 14 (2001) 791–798. <https://doi.org/10.1021/tx000156c>.
- [6] J. Devi, J. Yadav, *Anticancer Agents Med. Chem.* 18 (2018) 335–353. <https://doi.org/10.2174/1871520617666171106125114>.
- [7] M. Gielen, *Appl. Organomet. Chem.* 16 (2002) 481–494. <https://doi.org/10.1002/aoc.331>.
- [8] M. Giuliano, C. Pellerito, A. Celesia, T. Fiore, S. Emanuele, *Molecules*. 26 (2021) 5010. <https://doi.org/10.3390/molecules26165010>.
- [9] X.-X. Zhang, D.-S. Zhao, J. Wang, H. Zhou, L. Wang, J.-L. Mao, J.-X. He, *Pharm.* 76 (2021) 55–60. <https://doi.org/10.1691/ph.2021.0958>.
- [10] K. Zduńska, A. Dana, A. Kolodziejczak, H. Rotsztein, *Skin Pharmacol. Physiol.* 31 (2018) 332–336. <https://doi.org/10.1159/000491755>.
- [11] S. Emanuele, A. D’Anneo, G. Calvaruso, C. Cernigliaro, M. Giuliano, M. Lauricella, *Chem. Res. Toxicol.* 31 (2018) 201–210. <https://doi.org/10.1021/acs.chemrestox.7b00311>.
- [12] S.S. Damasceno, B.B. Dantas, J. Ribeiro-Filho, D. Antônio M Araújo, J. Galberto M da Costa, *Curr. Pharm. Des.* 23 (2017) 3015–3023. <https://doi.org/10.2174/1381612822666161208145508>.
- [13] C. Pellerito, S. Emanuele, F. Ferrante, A. Celesia, M. Giuliano, T. Fiore, *J. Inorg. Biochem.* 205 (2020) 110999. <https://doi.org/10.1016/j.jinorgbio.2020.110999>.
- [14] A. Celesia, O. Morana, T. Fiore, C. Pellerito, A. D’Anneo, M. Lauricella, D. Carlisi, A. De Blasio, G. Calvaruso, M. Giuliano, S. Emanuele, *Int. J. Mol. Sci.* 21 (2020) 8135. <https://doi.org/10.3390/ijms21218135>.

- [15] S. Emanuele, M. Lauricella, A. D'Anneo, D. Carlisi, A. De Blasio, D. Di Liberto, M. Giuliano, *Int. J. Mol. Sci.* 21 (2020) 5029. <https://doi.org/10.3390/ijms21145029>.
- [16] S. Emanuele, A. Celesia, A. D'Anneo, M. Lauricella, D. Carlisi, A. De Blasio, M. Giuliano, *Int. J. Mol. Sci.* 22 (2021) 7963. <https://doi.org/10.3390/ijms22157963>.
- [17] Y.-J. Li, Y.-H. Lei, N. Yao, C.-R. Wang, N. Hu, W.-C. Ye, D.-M. Zhang, Z.-S. Chen, *Chin. J. Cancer*. 36 (2017) 52. <https://doi.org/10.1186/s40880-017-0219-2>.
- [18] P. Robak, T. Robak, Bortezomib for the Drugs RD. 19 (2019) 73–92. <https://doi.org/10.1007/s40268-019-0269-9>.
- [19] J. Li, X. Zhang, J. Shen, J. Guo, X. Wang, J. Liu, *Mol. Med. Rep.* 20 (2019) 2410–2418. <https://doi.org/10.3892/mmr.2019.10467>.
- [20] L. Zhang, M. Wu, R. Su, D. Zhang, G. Yang, *Recent Patents Anticancer Drug Discov.* 16 (2021). <https://doi.org/10.2174/1574892816666211202154536>.
- [21] L.D. Fricker, *Annu. Rev. Pharmacol. Toxicol.* 60 (2020) 457–476. <https://doi.org/10.1146/annurev-pharmtox-010919-023603>.
- [22] G. LaPlante, W. Zhang, *Cancers*. 13 (2021) 3079. <https://doi.org/10.3390/cancers13123079>.
- [23] M.S.F. Roeten, J. Cloos, G. Jansen, *Cancer Chemother. Pharmacol.* 81 (2018) 227–243. <https://doi.org/10.1007/s00280-017-3489-0>.
- [24] D. Zagirova, R. Autenried, M.E. Nelson, K. Rezvani, *J. Cancer*. 12 (2021) 2472–2487. <https://doi.org/10.7150/jca.52414>.
- [25] T. Wu, W. Chen, Y. Zhong, X. Hou, S. Fang, C.-Y. Liu, G. Wang, T. Yu, Y.-Y. Huang, X. Ouyang, H.Q.X. Li, L. Cui, Y. Yang, *Mol. Cancer Ther.* 16 (2017) 717–728. <https://doi.org/10.1158/1535-7163.MCT-16-0553>.
- [26] I.Y. Kim, M.J. Shim, D.M. Lee, A.R. Lee, M.A. Kim, M.J. Yoon, M.R. Kwon, H.I. Lee, M.J. Seo, Y.W. Choi, K.S. Choi, *Biochem. Pharmacol.* 162 (2019) 41–54. <https://doi.org/10.1016/j.bcp.2018.12.006>.
- [27] G. Park, D. Jin, D. Kim, *Oncol. Lett.* (2018). <https://doi.org/10.3892/ol.2018.9233>.
- [28] J.H. Lee, K.-H. Jung, J.W. Park, S.H. Moon, Y.S. Cho, K.-H. Lee, *Sci. Rep.* 10 (2020) 14308. <https://doi.org/10.1038/s41598-020-71366-3>.
- [29] M. Lauricella, V. Lo Galbo, C. Cernigliaro, A. Maggio, A. Palumbo Piccionello, G. Calvaruso, D. Carlisi, S. Emanuele, M. Giuliano, A. D'Anneo, *Antioxidants*. 8 (2019) 422. <https://doi.org/10.3390/antiox8100422>.
- [30] A. Notaro, S. Sabella, O. Pellerito, R. Vento, G. Calvaruso, M. Giuliano, *Int. J. Oncol.* 48 (2016) 1039–1044. <https://doi.org/10.3892/ijo.2015.3307>.
- [31] G.B. Deacon, F. Huber, R.J. Phillips, *Inorganica Chim. Acta*. 104 (1985) 41–45. [https://doi.org/10.1016/S0020-1693\(00\)83783-4](https://doi.org/10.1016/S0020-1693(00)83783-4).
- [32] O. Pellerito, C. Prinzivalli, E. Foresti, P. Sabatino, M. Abbate, G. Casella, T. Fiore, M. Scopelliti, C. Pellerito, M. Giuliano, G. Grasso, L. Pellerito, *J. Inorg. Biochem.* 125 (2013) 16–25. <https://doi.org/10.1016/j.jinorgbio.2013.04.008>.
- [33] C. Pellerito, O. Morana, F. Ferrante, G. Calvaruso, A. Notaro, S. Sabella, T. Fiore, *J. Inorg. Biochem.* 150 (2015) 18–27. <https://doi.org/10.1016/j.jinorgbio.2015.06.001>.
- [34] Y.Q. Qu, F. Gordillo-Martinez, B.Y.K. Law, Y. Han, A. Wu, W. Zeng, W.K. Lam, C. Ho, S.W.F. Mok, H.Q. He, V.K.W. Wong, R. Wang, *Cell Death Dis.* 9 (2018) 361. <https://doi.org/10.1038/s41419-018-0397-0>.
- [35] M. Takenokuchi, K. Miyamoto, K. Saigo, T. Taniguchi, *Anticancer Res.* 35 (2015) 3307–3316.
- [36] M. Ri, , *Int. J. Hematol.* 104 (2016) 273–280. <https://doi.org/10.1007/s12185-016-2016-0>.
- [37] J. Zhang, Z. Ye, W. Chen, J. Culpepper, H. Jiang, L.E. Ball, S. Mehrotra, A. Blumental-Perry, K.D. Tew, D.M. Townsend, *Free Radic. Biol. Med.* 160 (2020) 755–767. <https://doi.org/10.1016/j.freeradbiomed.2020.09.013>.

- [38] F. Shirazi, R.J. Jones, R.K. Singh, J. Zou, I. Kuiatse, Z. Berkova, H. Wang, H.C. Lee, S. Hong, L. Dick, N. Chattopadhyay, R.Z. Orlowski, *Proc. Natl. Acad. Sci.* 117 (2020) 20004–20014. <https://doi.org/10.1073/pnas.2005052117>.
- [39] M. Ni, Y. Zhang, A.S. Lee, *Biochem. J.* 434 (2011) 181–188. <https://doi.org/10.1042/BJ20101569>.
- [40] A. Arena, M.A. Romeo, R. Benedetti, L. Masuelli, R. Bei, M.S. Gilardini Montani, M. Cirone, *Pharmaceuticals*. 14 (2021) 1068. <https://doi.org/10.3390/ph14111068>.
- [41] H. Ji, W. Wang, X. Li, X. Han, X. Zhang, J. Wang, C. Liu, L. Huang, W. Gao, *Pharmacol. Res.* (2021) 106013. <https://doi.org/10.1016/j.phrs.2021.106013>.