Investigational New Drugs (2019) 37:27–34
https://doi.org/10.1007/s10637-018-0594-9

PRECLINICAL STUDIES

Copper-tolfenamic acid: evaluation of stability and anti-cancer activity

Myrna Hurtado[1] · Umesh T. Sankpal[2] · Jaya Chhabra[3] · Deondra T. Brown[3] · Rajasekhar Maram[2] · Rafid Patel[2] · Raj K. Gurung[3] · Jerry Simecka[4] · Alvin A. Holder[3] · Riyaz Basha[1,2,4]

Received: 13 February 2018 / Accepted: 21 March 2018 / Published online: 15 May 2018

Summary

The non-steroidal anti-inflammatory drug, Tolfenamic acid (TA) acts as an anti-cancer agent in several adult and pediatric cancer models. Copper (Cu) is an important element with multiple biological functions and has gained interest in medical applications. Recently, $[Cu(TA)_2(bpy)]$ (Cu-TA) has been synthesized in order to enhance therapeutic activity. In this study, we synthesized Cu-TA using an established method, characterized it by UV visible spectroscopy and Fourier-transform infrared spectroscopy (FTIR), and tested its anti-cancer activity using twelve cell lines representing various cancers, such as Ewing sarcoma, glioblastoma, medulloblastoma, neuroblastoma, pancreatic and prostate. The anti-proliferative activity of Cu-TA was determined at 48 h post-treatment and compared with the parental compound, TA. The IC_{50} values were calculated using GraphPad Prism software. The biological stability of Cu-TA was evaluated using twelve-month-old powder and six-month-old stock solution. Cardiomyocytes (H9C2) were used to test the cytotoxicity in non-malignant cells. Cu-TA showed higher anti-proliferative activity, and the IC_{50} values were 30 to 80% lower when compared with TA. H9C2 cells were non-responsive to Cu-TA, suggesting that it is selective towards malignant cells. Comparison of the twelve-month-old powder and six-month-old stock solution using the Panc1 cell line showed similar IC_{50} values (<5% variation), confirming the stability of Cu-TA either in powder or solution form. These findings demonstrate the potential of Cu-TA as an effective anti-cancer agent. Further studies to delineate the detailed mechanism of action of Cu-TA for specific cancer model are underway.

Keywords Copper-Tolfenamic acid · Survivin · Specificity protein 1 · Cardiomyocytes

Introduction

Metals are known to play a crucial role in the sustainability of life, and the homeostasis of metals is important for maintaining good health. Particularly, the essential metals critically involved in multiple biological functions and their use in medical benefits have been explored for a longtime [1, 2]. Even non-essential metals show medical benefits and are used in some medicines. For example, platinum (Pt)-based drugs such as cisplatin and carboplatin are routinely used in cancer treatment [3–7]. Copper (Cu) is a transition element and an essential metal that plays a critical role in biological functions such as growth, respiration and metabolism [1, 2]. The medical use of Cu has gained substantial interest, resulting in thousands of publications covering several diseases. Copper-containing compounds have been tested for their ability to potentiate the therapeutic response of certain medicines, and this work has led to a strategy for testing Cu-based drugs. Subsequently, Cu complexes were tested in pre-clinical studies using cancer models including in targeted delivery [8–10].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are increasingly being examined for cancer prevention and treatment (either alone or with chemo or radiation therapy) using various cancer models. NSAIDs are not only effective, but less toxic than conventional chemotherapy drugs [11, 12]. However, there is insufficient clinical evidence to replace current standard care consisting of chemotherapy and/or radiation with NSAIDs or other small molecules. Other limitations of using NSAIDs in cancer therapy are morbidities associated with long-term use of COX inhibitors [13, 14]. Extended use

✉ Riyaz Basha
Riyaz.Basha@unthsc.edu; riyazbasha@yahoo.com

[1] Graduate School of Biomedical Sciences, Fort Worth, TX, USA

[2] Texas College of Osteopathic Medicine, UNT Health Science Center, 3500 Camp Bowie Blvd, Fort Worth, TX 76107, USA

[3] Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, VA 23529, USA

[4] Pre-clinical Services, UNT Systems College of Pharmacy, UNT Health Science Center, Fort Worth, TX 76107, USA

of COX-1 inhibitors can cause gastrointestinal issues, while COX-2 inhibitors can increase the incidence of cardiovascular events. Therefore, there is a rising interest in finding NSAIDs that work in mechanisms independent of COX inhibition.

Tolfenamic acid (TA) is an NSAID and is used as a generic drug for treating migraine headaches in Europe. Studies from our group and others demonstrate that TA contains anti-cancer properties, such as inhibition of cancer cell and tumor growth in pre-clinical models. TA works through downregulation of transcription factors, Specific proteins 1 and 3 (Sp1 and Sp3), and an inhibitor of apoptosis protein, baculoviral inhibitor of apoptosis repeat-containing 5 (survivin). Sp1, Sp3 and survivin are found to be over-expressed in several cancers and associated with aggressive disease and its resistance to standard treatment options [15–19]. Importantly, TA was approved for Phase I clinical trials alongside gemcitabine and radiation for pancreatic cancer.

Metal complexes in metallodrugs have a synergistic activity that optimizes the parent drug's bioactivity [20, 21]. Recently, metal complexes of NSAIDs were shown to be more effective compared with regular NSAIDs [22]. The Copper(II)-Tolfenamic acid (Cu-TA) complex was synthesized and found to contain a higher antioxidant activity compared to TA [23]; however, its anti-cancer activity needs further study. The objective of this investigation is to determine the effectiveness of Cu-TA for inducing anti-proliferative activity in cancer cells. In this screening study, we have used twelve cells lines representing six malignancies that affects children, adolescent/young adults and adults. The selected cancers are, Ewing sarcoma, glioblastoma, medulloblastoma, neuroblastoma, pancreatic and prostate cancers. The data presented in this study includes the characterization and biological stability testing of the compound as well as the assessment of anti-proliferative activity against cancer cells and cardiomyocytes (non-malignant cells). The possible mechanisms associated with this compound, as determined from existing literature, are also schematically emphasized.

Methods

Characterization of Cu-TA

The Cu-TA complex [$Cu(TA)_2(bpy)$] was prepared following published methodology (bpy - 2,2'-bipyridine) [23]. Then, the complex was characterized using UV-visible and FTIR spectroscopic techniques. Bruker Platinum ATR-IR spectrometer was used for FTIR spectroscopy, and the UV-visible spectrum was acquired using an Agilent HP8453 diode array spectrophotometer [24]. Using the FTIR spectrum, the two important stretching frequencies were observed at 1579 cm^{-1} and 1385 cm^{-1} for CO_2(asym) and CO_2(sym), respectively for the coordinated carboxlate anion of TA. In the UV-visible spectrum (with DMSO as a solvent), the λ_{max} was observed at 680 nm, which was assigned as a d-d transition due to the presence of the Cu(II) metal center.

Preparation of stock concentrations

TA and Cu-TA were dissolved in dimethyl sulfoxide (DMSO), and 10-mM stock solutions were prepared. For dose curves, serial dilutions were made in cell culture media corresponding to each cell line.

Cell lines and culture conditions

Twelve human cancer cells lines representing 6 diseases were used in this study. Ewing sarcoma (CHLA10 and TC205), glioblastoma (A172 and U118), medulloblastoma (D283 and DAOY), neuroblastoma (SH SY-5Y and LA155n), pancreatic (MIA PaCa-2 and Panc1) and prostate (PC3 and LANCaP) cell lines were grown following established cell culture conditions in media; Dulbecco's Modified Eagle Medium (MIA PaCa-2, and Panc1), Eagle's Minimum Essential Medium (D283 and DAOY and Roswell Park Memorial Institute (SH SY-5Y, LA155n, PC3, and LANCaP), Iscove's Modified Dulbecco's Medium (CHLA10, TC 205) supplemented with fetal bovine serum and 1% penicillin streptomycin were used following optimum conditions provided by the suppliers' instructions or published work. Cells were maintained in an incubator at 37 °C with 5% CO_2.

Cell viability

Selected cell lines were seeded in 96-well plates; each well contained 4000 cells, except DAOY cells. For DAOY (due to itslarger size), only 2000 cells/well were seeded. Cells were treated with DMSO (vehicle) for the control or increasing concentrations of TA (5/10/25/50/100 μM) or Cu-TA (5/10/25/50 μM). After 48 h post treatment, cells were incubated with CellTiter-Glo reagent in the dark for 20 min. Then, the cell viability was measured by detecting luminescence using the Synergy HT (BioTek) plate reader [19, 25].

Statistical analysis

Results were expressed as mean ± SD. Data were statistically analyzed (one-way ANOVA) and the significance between treated and untreated cells were evaluated. Samples with *p*-values <0.05 were considered significant.

Results

Characterization and stability of Cu-TA complex Physical characterization and stability of Cu-TA was performed using

FTIR and UV-visible spectroscopies. Both spectra were obtained with the freshly prepared Cu-TA powder and 8 months later. As shown in Fig. 1, both samples exhibit very similar spectra, confirming the stability of the complex in powder form. In the freshly prepared powder's FTIR spectrum, the stretching frequencies were observed at 1579 cm^{-1} and 1385 cm^{-1} for CO_2(asym) and CO_2(sym), respectively, while in the 8-month-old powder, the stretching frequencies were observed at 1578 cm^{-1} and 1385 cm^{-1}. The UV-visible spectrum (λ_{ma} at 680 nm) of the fresh and 8-month-old compound (in powder form) exhibited molar extinction coefficient values of 92 M^{-1} cm^{-1} and 89 M^{-1} cm^{-1}, respectively.

Anti-proliferative activity of Cu-TA against human cancer cell lines Twelve cancer cell lines representing 6 human cancers were evaluated to determine the anti-proliferative activity of both TA and Cu-TA. Dose curves are shown in Fig. 2, and IC_{50} values were determined (Fig. 3) and compared among TA and Cu-TA. The results are summarized in Table 1. IC_{50} values for Cu-TA were much lower than TA. For example, in Ewing sarcoma cells, Cu-TA's IC_{50} values were 68.6% and 48.3% less than TA, respectively, for CHLA10 and TC205 cells. Similar trends were observed in other cancer models. Glioblastoma (A172: 73.1%; U118: 85.5%), medulloblastoma (DAOY: 71.9%; D283:56.3%), neuroblastoma (SH-SY5Y: 47.1%; LA155n: 80.4%), pancreatic (MIA PaCa-2: 48.7%; Panc1: 56.9%) and prostate (LNCap: 46.8%; PC3: 32.8%) cancer cell lines also showed IC_{50} values less than TA, as indicated in the parentheses.

Assessing the stability of Cu-TA in anti-proliferative activity Biological activity testing of the Cu-TA complex was performed using the 6-month-old and one-year-old Cu-TA using pancreatic cancer cells, Panc1. Dose curves were repeated using 12-month-old Cu-TA powder and 6-month-old solution. The dose-response curves were plotted. Both the 6- and 12-month-old compounds showed a similar trend in cell growth inhibition against the pancreatic cancer cell line Panc1, and

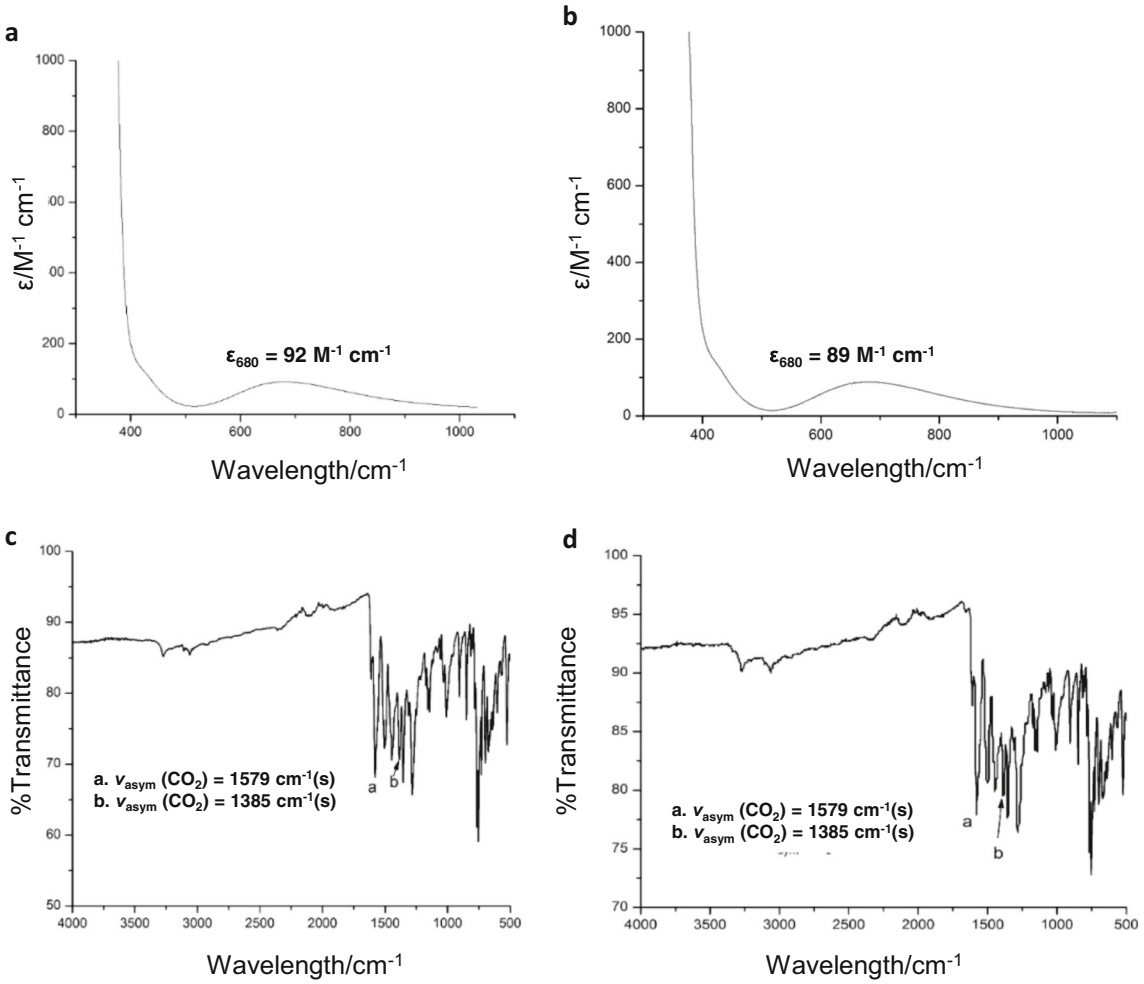

Fig. 1 Characterization of Cu-TA: Physical characterization and stability of Cu-TA was performed using (**a** & **b**) UV-visible spectroscopy and (**c** & **d**) FTIR spectroscopy. Tests were performed with (**a** &**c**) freshly prepared compound and (**b** & **d**) 6-month old compound (powder) stored at room temperature

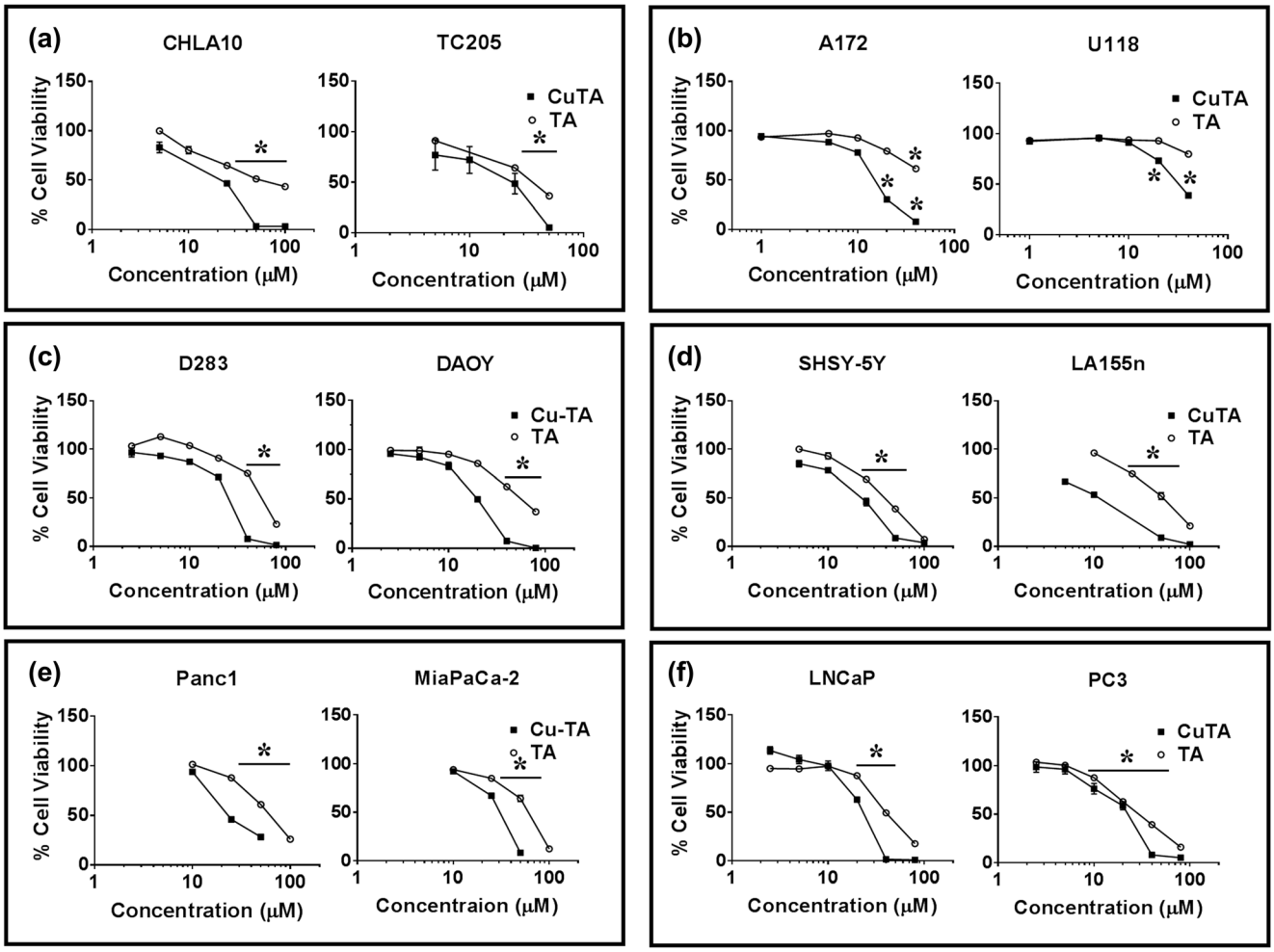

Fig. 2 Anti-proliferative activity of Cu-TA against cancer cell lines: Twelve cancer cell lines representing (**a**) Breast cancer, (**b**) Glioblastoma, (**c**) Medulloblastoma, (**d**) Neuroblastoma, (**e**) Pancreatic cancer and (**f**) Prostate cancer were treated with increasing concentrations of Cu-TA or TA and the cell growth was measured after 48 h. Dose curves were plotted using GraphPad Prism V6.0. Each data point is mean ± SD of triplicate samples: '*' indicates significance (p-value <0.05) at the indicated data point for single cell line. The 'line and *' indicate significance (p-value <0.05) at the data points covering the marked area for both cell lines

the IC_{50} values were found to be within the margin of error (<5% variation). These results confirm stability in the biological activity of the compound (Fig. 4a&b).

Effect of Cu-TA against non-malignant cells To determine the effect of Cu-TA against non-cancerous cells, we evaluated the cytotoxicity of the compound on cardiomyocytes, H9C2. Cells were treated with increasing dosages of TA or Cu-TA, and cell viability was measured at 24 and 48 h (Fig. 4c). H9C2 cells growth was not affected by Cu-TA, suggesting that Cu-TA is not toxic against cardiomyocytes.

Discussion

Cancer treatment involves cytotoxic agents that often cause severe side-effects. Even though the outcomes of cancer patients have tremendously improved over the past decades, prognoses of certain types of cancers (e.g., glioblastoma, pancreatic cancer) are still extremely low. Therefore, identifying new investigational agents with anti-cancer activity and low toxic effects on normal/healthy cells is extremely important. Recently, preclinical studies demonstrate significant progress in identifying small molecules, such as NSAIDs, to use in cancer treatment. TA is an NSAID which was tested in multiple cancer models by many research groups. In addition, TA was approved for use in Phase I clinical trials with pancreatic cancer patients (in conjunction with gemcitabine/radiation). Even though TA is a relatively safer NSAID, its IC_{50} values may elicit concerns in certain cancer models. Therefore, the objective of this study was to test Cu-TA for enhanced anti-cancer activity.

The Cu-TA was successfully synthesized using an established protocol [23] and characterized to ascertain structural confirmation. The physical characterization of the Cu-TA

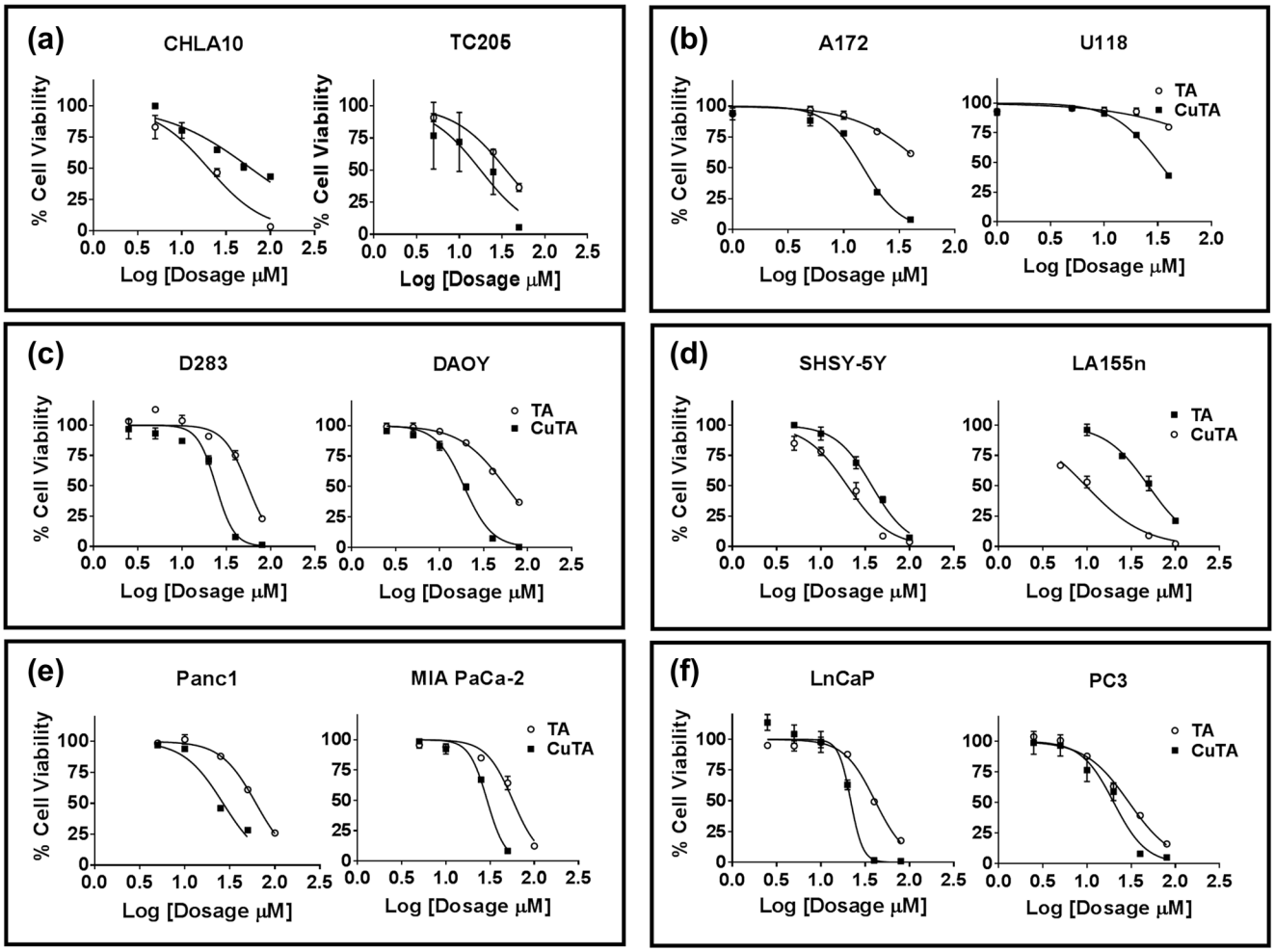

Fig. 3 Effect of Cu-TA on cancer cell viability and determination of IC_{50} values. Indicated cancer cell lines were treated with TA or Cu-TA and the dose curves were plotted using the log values. Each data point is mean ± SD of triplicate samples

Table 1 IC_{50} of Cu-TA compared to TA in various human cancer cell lines: Ewing sarcoma, glioblastoma, medulloblastoma, neuroblastoma, pancreatic and prostate cancer cell lines were treated with increasing concentrations of TA or Cu-TA and the dose curves were plotted using the log values and IC_{50} values were calculated

Cancer	Cell line	Cu-TA μM	TA μM	Percent decrease over TA
Ewing Sarcoma	CHLA10	18.88	60.22	68.64
	TC205	17.73	34.31	48.32
Glioblastoma	A172	15.05	56.11	73.18
	U118	32.31	223	85.51
Medulloblastoma	D283	24.15	55.3	56.33
	DAOY	23.12	82.22	71.88
Neuroblastoma	SH-SY5Y	19.96	37.7	47.06
	LA155n	9.73	49.62	80.39
Pancreatic	MIA PaCa-2	29.32	57.2	48.74
	Panc1	26.65	61.76	56.85
Prostate	LNCaP	21.75	40.9	46.82
	PC3	20.1	29.9	32.78

Percent change of Cu-TA's IC_{50} over TA's IC_{50} was calculated for all cell lines. Each data point is mean of triplicate samples

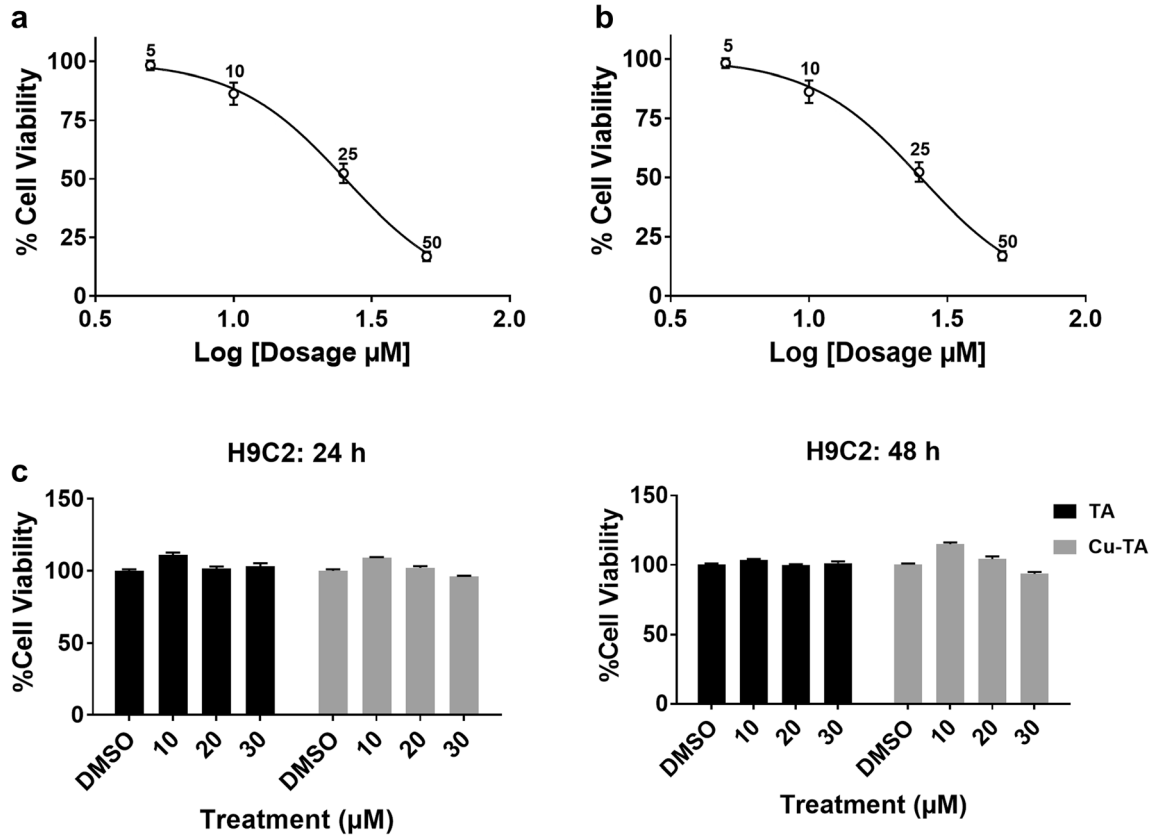

Fig. 4 Assessing the biological stability of Cu-TA in pancreatic cells and cytotoxicity in non-malignant cells. The biological activity stability of the compound was tested using (**a**) one-year old powder and (**b**) six-month old solution of the Cu-TA complex. Panc1 cells were treated with increasing dosages of Cu-TA for 48 h. The IC_{50} values were calculated using GraphPad Prism V6.0. H9C2 cells were also treated with increasing dosages of (**c**) Cu-TA or TA and cell viability was measured at 24 and 48 h post-treatment. Each data point is mean ± SD of triplicate samples. *: *p*-value <0.05

complex was performed again after 8 months, and both results were found to be consistent as evaluated by their FTIR and UV visible spectra. Importantly, the anti-cancer activity of Cu-TA in its powder form was retained through 12 months after synthesis. Therefore, the Cu-TA complex is physically and biologically stable for at least a year.

Cu-TA had significantly greater activity against different cancer cell growth than TA. The anti-proliferative activity of Cu-TA was evaluated using twelve cancer cell lines. The cancer cell lines for this study were selected representing the cancers that are diagnosed in children (medulloblastoma and neuroblastoma), adolescent and young adults (Ewing sarcoma) and adults (glioblastoma, pancreatic and prostate). Cu-TA caused growth inhibition in all cancer cell lines and showed a significantly higher response when compared with TA. The IC_{50} values for some cancer cell lines (e.g., glioblastoma, medulloblastoma, neuroblastoma) were > 70% lower than TA. Even though we cannot think of any similarity among these cell lines, it seems that multiple mechanisms could be associated with such a response (Fig. 5). Cu possesses pro-oxidant activity and induces reactive oxygen species, which leads to cytotoxicity [26]. Cu (II) ions are known to cause cytotoxicity

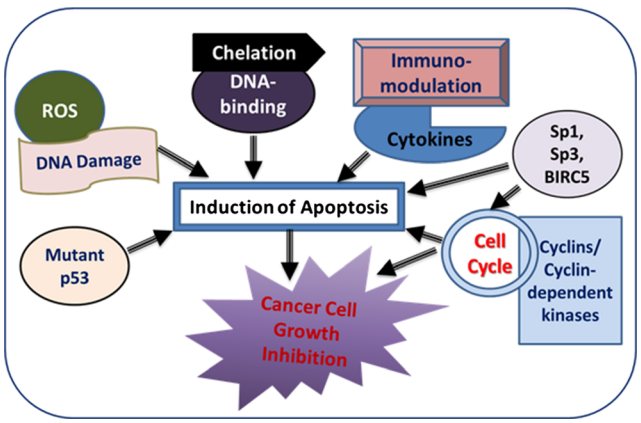

Fig. 5 The potential mechanisms that may cause the anti-cancer activity of Cu-TA: Exploring the published work on Cu(II)-containing products in cancer, it is postulated that multiple mechanisms could be associated with the anti-cancer activity of Cu-TA. For example, induction of Reactive Oxygen Species (ROS), chelating effect disrupting the DNA-binding, immunomodulation (eg., cytokines) can induce apoptosis. Cu compounds are also known to suppress the activity of mutated p53. Inhibition of Sp1, Sp3 and survivin can cause both induction of apoptosis and cell cycle arrest, while the direct/in-direct effect on cyclins and/or cyclin-dependent kinases can lead to cell cycle arrest ultimately leading to cell death or growth inhibition

in cancer cells by interfering with iron-sulfur clusters in proteins and conformational changes in metallo-proteins by displacing metals such as Zinc [27–29]. Copper(II) complexes with the ligands such as bis(thiosemicarbazone) were tested in several cancer models [30–32], while CuII(gtsm) [glyoxalbis(N4methylthiosemicarbazonato)CuII] was very effective at reducing cancer burden and lesion grade in an animal model (TRAMP) for prostate cancer [26]. Zhang et al. demonstrated that copper (II) complexe (3-indolecarboxylic acid) targets the 20S proteasome at the β5 subunit and cause the deactivation of the proteasome in cancer cells as well as anti-cancer activity [33]. (II)-containing species are believed to act as anti-inflammatory agents [31, 34–36] by modulating the synthesis of prostaglandins [37–39]. TA is known to inhibit Sp1, Sp3 and survivin, and these properties may be either retained or even improved with Cu-TA. These proteins participate in multiple impactful activities that can induce apoptosis and/or cell cycle arrest. Cu(II)-containing products can also exhibit immunomodulatory responses that affect the expression of cytokines (e.g., interleukin IL-2) [40]. Thus, the addition of Cu enhances the cytotoxic activity of TA against cancer cell proliferation.

Neither TA nor Cu-TA caused any significant cytotoxicity in non-malignant H9C2 cells. These results are very crucial and confirm that the anti-proliferative activity of Cu-TA is not an obvious effect against all cells. NSAIDs are known to induce cardiotoxicity, and these results showed no toxicity in cardiomyocytes. This suggests that this compound is relatively safe; however, further experiments using more non-malignant cells and animal studies are required to confirm the safety of Cu-TA.

Formation of stable metal NSAIDs with anti-cancer activity is a challenge, and several compounds were made and tested with little to moderate success due to limitations in stability, specificity and consistent biological (anti-cancer) activity. This investigation demonstrates the preparation of a Cu-TA complex with long-term stability in physical structure and activity. Interestingly, Cu-TA was more effective than its parent compound against all tested cancer cell lines; in particular, it exhibited significantly higher anti-proliferative activity with 50–80% decrease in the IC_{50} values. Further experiments are underway using cancer models to precisely understand the underlying mechanisms of action of this compound.

Acknowledgements Authors thank the Children's Oncology Group (COG) for freely providing the Ewing sarcoma cell lines, CHLA10 and TC205. Authors also acknowledge the technical assistance of Ms. Lina Albeer for culturing COG cell lines.

Funding Some of the research presented in this manuscript is partially supported by a grant from the Shirley E. Noland Foundation, Melbourne, FL, USA awarded to RB. UTS and RB are supported by a grant (# 2 U54 MD006882–06) from the National Institutes of Minority Health and Health Disparities. RM is supported by an International Fellowship from the Indian Council of Medical Research.

Compliance with ethical standards

Conflicts of interest M Hurtado declares that she has no conflict of interest. U T Sankpal declares that he has no conflict of interest. Jaya Chhabra declares that she has no conflict of interest. Deondra T. Brown declares that she has no conflict of interest. Rafid Patel declares that he has no conflict of interest. Raj K. Gurung declares that he has no conflict of interest. Jerry Simecka declares that he has no conflict of interest. Alvin A. Holder declares that he has no conflict of interest. Riyaz Basha declares that he has no conflict of interest.

Ethical approval This manuscript does not contain any experiments with human subjects or animals conducted by any of the contributing authors.

Informed consent It is a preclinical study. Therefore, formal consent is not required for this type of study.

References

1. Puig S, Thiele DJ (2002) Molecular mechanisms of copper uptake and distribution. Curr Opin Chem Biol 6(2):171–180
2. Szymanski P, Fraczek T, Markowicz M, Mikiciuk-Olasik E (2012) Development of copper based drugs, radiopharmaceuticals and medical materials. Biometals 25(6):1089–1112. https://doi.org/10.1007/s10534-012-9578-y
3. Douple EB, Richmond RC, O'Hara JA, Coughlin CT (1985) Carboplatin as a potentiator of radiation therapy. Cancer Treat Rev 12(Suppl A):111–124
4. Einhorn LH, Williams SD (1979) The role of cis-platinum in solid-tumor therapy. N Engl J Med 300(6):289–291. https://doi.org/10.1056/NEJM197902083000605
5. Long DF, Repta AJ (1981) Cisplatin: chemistry, distribution and biotransformation. Biopharm Drug Dispos 2(1):1–16
6. Wagstaff AJ, Ward A, Benfield P, Heel RC (1989) Carboplatin. A preliminary review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the treatment of cancer. Drugs 37(2):162–190
7. Woloschuk DM, Pruemer JM, Cluxton RJ, Jr. (1988) Carboplatin: a new cisplatin analog. Drug Intell Clin Pharm 22 (11):843–849
8. Pramanik A, Laha D, Dash SK, Chattopadhyay S, Roy S, Das DK, Pramanik P, Karmakar P (2016) An in-vivo study for targeted delivery of copper-organic complex to breast cancer using chitosan polymer nanoparticles. Mater Sci Eng C Mater Biol Appl 68:327–337. https://doi.org/10.1016/j.msec.2016.05.014
9. Allensworth JL, Evans MK, Bertucci F, Aldrich AJ, Festa RA, Finetti P, Ueno NT, Safi R, McDonnell DP, Thiele DJ, Van Laere S, Devi GR (2015) Disulfiram (DSF) acts as a copper ionophore to induce copper-dependent oxidative stress and mediate anti-tumor efficacy in inflammatory breast cancer. Mol Oncol 9(6):1155–1168. https://doi.org/10.1016/j.molonc.2015.02.007
10. Pramanik A, Laha D, Pramanik P, Karmakar P (2014) A novel drug "copper acetylacetonate" loaded in folic acid-tagged chitosan nanoparticle for efficient cancer cell targeting. J Drug Target 22(1):23–33. https://doi.org/10.3109/1061186X.2013.832768
11. Rayburn ER, Ezell SJ, Zhang R (2009) Anti-inflammatory agents for Cancer therapy. Mol Cell Pharmacol 1(1):29–43. https://doi.org/10.4255/mcpharmacol.09.05
12. Thun MJ, Henley SJ, Patrono C (2002) Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. J Natl Cancer Inst 94(4):252–266

13. Solomon DH, Husni ME, Libby PA, Yeomans ND, Lincoff AM, Lupsilonscher TF, Menon V, Brennan DM, Wisniewski LM, Nissen SE, Borer JS (2017) The risk of major NSAID toxicity with celecoxib, ibuprofen, or naproxen: a secondary analysis of the PRECISION trial. Am J Med 130(12):1415–1422 e1414. https://doi.org/10.1016/j.amjmed.2017.06.028
14. Walker C, Biasucci LM (2018) Cardiovascular safety of non-steroidal anti-inflammatory drugs revisited. Postgrad Med 130(1): 55–71. https://doi.org/10.1080/00325481.2018.1412799
15. Abdelrahim M, Baker CH, Abbruzzese JL, Safe S (2006) Tolfenamic acid and pancreatic cancer growth, angiogenesis, and Sp protein degradation. J Natl Cancer Inst 98(12):855–868. https://doi.org/10.1093/jnci/djj232
16. Liu X, Abdelrahim M, Abudayyeh A, Lei P, Safe S (2009) The nonsteroidal anti-inflammatory drug tolfenamic acid inhibits BT474 and SKBR3 breast cancer cell and tumor growth by repressing erbB2 expression. Mol Cancer Ther 8(5):1207–1217. https://doi.org/10.1158/1535-7163.MCT-08-1097
17. Pathi S, Li X, Safe S (2014) Tolfenamic acid inhibits colon cancer cell and tumor growth and induces degradation of specificity protein (Sp) transcription factors. Mol Carcinog 53(Suppl 1):E53–E61. https://doi.org/10.1002/mc.22010
18. Zhang X, Min KW, Liggett J, Baek SJ (2013) Disruption of the transforming growth factor-beta pathway by tolfenamic acid via the ERK MAP kinase pathway. Carcinogenesis 34(12):2900–2907. https://doi.org/10.1093/carcin/bgt250
19. Konduri S, Colon J, Baker CH, Safe S, Abbruzzese JL, Abudayyeh A, Basha MR, Abdelrahim M (2009) Tolfenamic acid enhances pancreatic cancer cell and tumor response to radiation therapy by inhibiting survivin protein expression. Mol Cancer Ther 8(3):533–542. https://doi.org/10.1158/1535-7163.MCT-08-0405
20. Katsarou ME, Efthimiadou EK, Psomas G, Karaliota A, Vourloumis D (2008) Novel copper(II) complex of N-propyl-norfloxacin and 1,10-phenanthroline with enhanced antileukemic and DNA nuclease activities. J Med Chem 51(3):470–478. https://doi.org/10.1021/jm7013259
21. Tan SJ, Yan YK, Lee PP, Lim KH (2010) Copper, gold and silver compounds as potential new anti-tumor metallodrugs. Future Med Chem 2(10):1591–1608. https://doi.org/10.4155/fmc.10.234
22. Manso Y, Comes G, Hidalgo J, Bush AI, Adlard PA (2011) Copper modulation as a therapy for Alzheimer's disease? Int J Alzheimers Dis 2011:370345. https://doi.org/10.4061/2011/370345
23. Tarushi A, Perontsis S, Hatzidimitriou AG, Papadopoulos AN, Kessissoglou DP, Psomas G (2015) Copper(II) complexes with the non-steroidal anti-inflammatory drug tolfenamic acid: structure and biological features. J Inorg Biochem 149:68–79. https://doi.org/10.1016/j.jinorgbio.2015.02.019
24. Altaf AA, Hashmat U, Yousaf M, Lal B, Ullah S, Holder AA, Badshah A (2016) Synthesis and characterization of azo-guanidine based alcoholic media naked eye DNA sensor. R Soc Open Sci 3(11):160351. https://doi.org/10.1098/rsos.160351
25. Shelake S, Sankpal UT, Paul Bowman W, Wise M, Ray A, Basha R (2017) Targeting specificity protein 1 transcription factor and survivin using tolfenamic acid for inhibiting Ewing sarcoma cell growth. Investig New Drugs 35(2):158–165. https://doi.org/10.1007/s10637-016-0417-9
26. Cater MA, Pearson HB, Wolyniec K, Klaver P, Bilandzic M, Paterson BM, Bush AI, Humbert PO, La Fontaine S, Donnelly PS, Haupt Y (2013) Increasing intracellular bioavailable copper selectively targets prostate cancer cells. ACS Chem Biol 8(7): 1621–1631. https://doi.org/10.1021/cb400198p
27. Denoyer D, Masaldan S, La Fontaine S, Cater MA (2015) Targeting copper in cancer therapy: 'Copper that Cancer'. Metallomics 7(11): 1459–1476. https://doi.org/10.1039/c5mt00149h
28. Festa RA, Thiele DJ (2011) Copper: an essential metal in biology. Curr Biol 21(21):R877–R883. https://doi.org/10.1016/j.cub.2011.09.040
29. Xiao Y, Chen DI, Zhang X, Cui Q, Fan Y, Bi C, Dou QP (2010) Molecular study on copper-mediated tumor proteasome inhibition and cell death. Int J Oncol 37(1):81–87
30. Barry VC, Conalty ML, O'Sullivan JF (1966) Anticancer activity of bisthiosemicarbazones of methylglyoxal. Cancer Res 26(10):2165–2168
31. French FA, Freedlander BL (1958) Carcinostatic action of polycarbonyl compounds and their derivatives. IV. Glyoxal bis (thiosemicarbazone) and derivatives. Cancer Res 18(11): 1290–1300
32. Petering HG, Buskirk HH, Underwood GE (1964) The anti-tumor activity of 2-Keto-3-Ethoxybutyraldehyde Bis(Thiosemicarbazone) and related compounds. Cancer Res 24:367–372
33. Zhang Z, Wang H, Wang Q, Yan M, Wang H, Bi C, Sun S, Fan Y (2016) Anticancer activity and computational modeling of ternary copper (II) complexes with 3-indolecarboxylic acid and 1,10-phenanthroline. Int J Oncol 49(2):691–699. https://doi.org/10.3892/ijo.2016.3542
34. Sorenson JR (1976) Copper chelates as possible active forms of the antiarthritic agents. J Med Chem 19(1):135–148
35. Sorenson JR (1987) Biology of copper complexes. Biology of copper complexes. Humana Press, Clifton
36. Sorenson JR (1989) Copper complexes offer a physiological approach to treatment of chronic diseases. Prog Med Chem 26:437–568
37. Franco L, Doria D (1997) Prostaglandins and nitric oxide in copper-complex mediated protection against ethanol-induced gastric damage. Pharmacol Res 36(5):395–399. https://doi.org/10.1006/phrs.1997.0247
38. Sakuma S, Fujimoto Y, Kitao A, Sakamoto H, Nishida H, Fujita T (1999) Simultaneous measurement of prostaglandin and arachidonoyl CoA formed from arachidonic acid in rabbit kidney medulla microsomes: the roles of Zn2+ and Cu2+ as modulators of formation of the two products. Prostaglandins Leukot Essent Fatty Acids 61(2):105–112. https://doi.org/10.1054/plef.1999.0078
39. Sakuma S, Fujimoto Y, Miyata Y, Ohno M, Nishida H, Fujita T (1996) Effects of Fe(2+), Zn(2+), cu(2+) and se(4+) on the synthesis and catabolism of prostaglandins in rabbit gastric antral mucosa. Prostaglandins Leukot Essent Fatty Acids 54(3):193–197
40. Hopkins RG, Failla ML (1999) Transcriptional regulation of interleukin-2 gene expression is impaired by copper deficiency in Jurkat human T lymphocytes. J Nutr 129(3):596–601