Supplementary file

Article

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Abstract: Piplartine (**1**) is an alkamide extracted from plants of the genus *Piper* and shows several pharmacological properties including antitumor activity. To improve this activity a series of analogues based on **1** have been synthesized by esterification and amidation using the 3,4,5-trimethoxycinnamic acid like starting material. In study, the moieties 3-(3,4,5-trimethoxyphenyl)acrylate and 3-(3,4,5-trimethoxyphenyl)acrylamide were maintained on esters and amides respectively. Meanwhile, functional changes were exploited and it was revealed that the presence of two aromatic rings in side-chain was important to improve the cytotoxic activity against U87MG cell line, such as the compound (*E*)-benzhydryl 3-(3,4,5-trimethoxyphenyl)acrylate (**10**), an ester that exhibited strong cytotoxicity and similar potency to paclitaxel, a positive control. Compound **10** had a marked concentration-dependent inhibitory effect on viability of U87MG cell line with apoptotic and oxidative processes, showing good potential for altering main molecular pathways, preventing tumor development. Moreover, it has strong bioavailability with non-genotoxic and non-cytotoxic properties on human blood cells. In a conclusion, the findings of the present study demonstrated that compound **10** could be a promising agent that may find applications in diseases associated with oxidative stress and as a prototype for the developing of novel drugs used in treatment of glioblastoma.

Keywords: cancer; cytotoxic activity; antitumor; phenylpropanoid; alkaloid; natural product; esters; oxidative stress; *Piper*; synthetic derivatives.

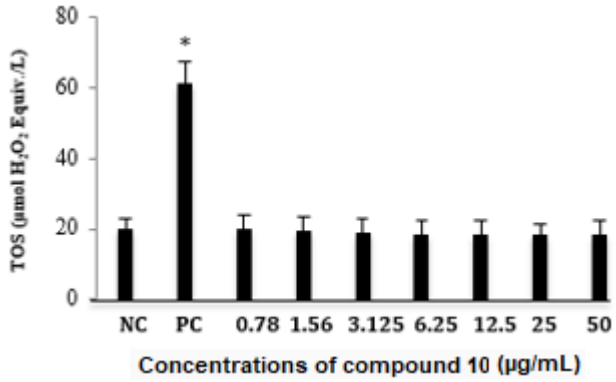


Figure 1- The effects of compound **10** on the levels of total oxidant status (TOS) in cultured U87MG cells48 h. NC: Negative control, PC: Positive control (hydrogen peroxide, 25 μM). $p< 0.05$, comparison to NC.

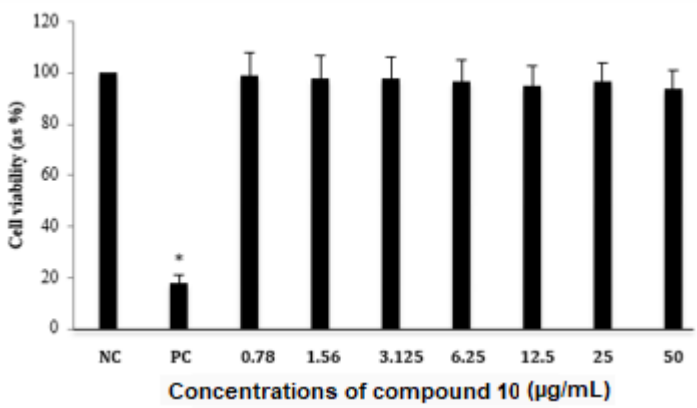


Figure 2- The *in vitro* effects of compound **10** on the cell viability of cultured human blood cells (MTT assay). Abbreviations are as in figure 2.

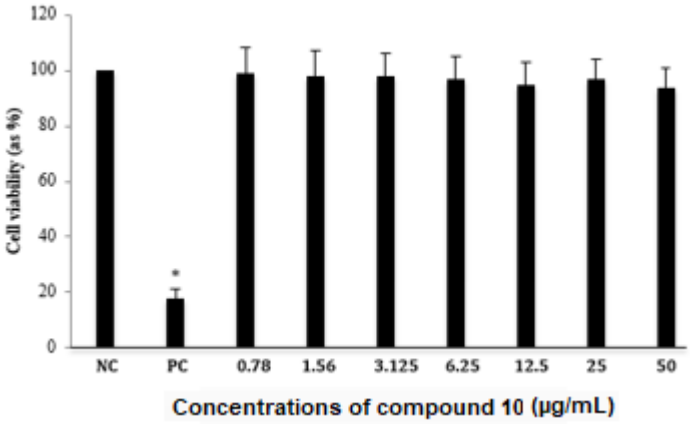


Figure 3- The *in vitro* effects of compound **10** on the cell viability of cultured human blood cells (LDH assay). Abbreviations are as in figure 2.

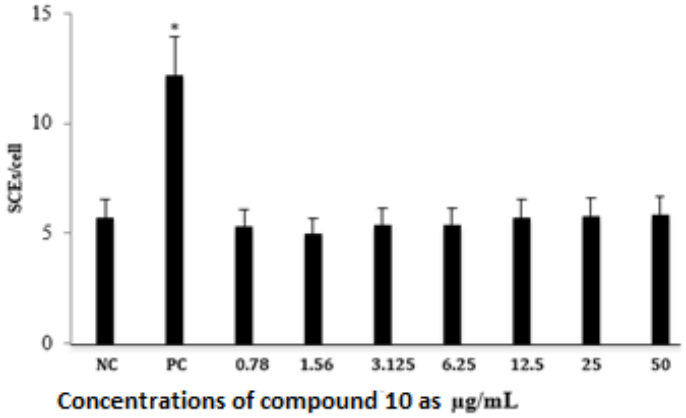
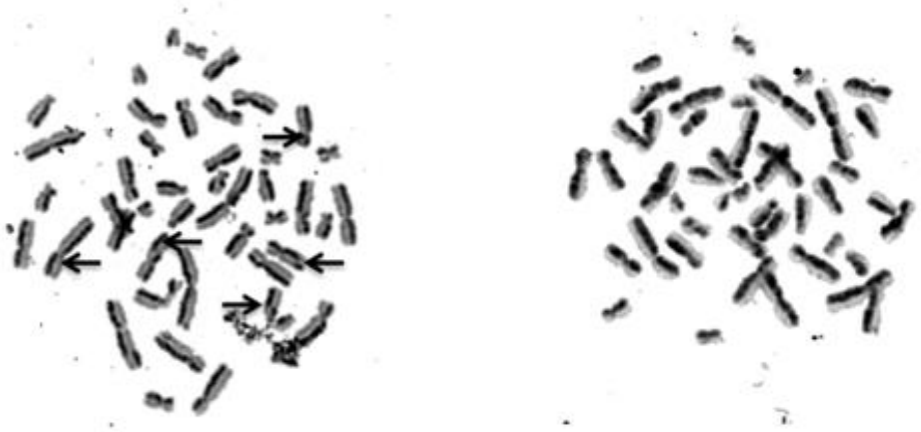


Figure 4- The *in vitro* effects of compound **10** on SCE rates in cultured human whole blood cells. NC: Negative control, PC: Positive control (Mitomycin-C, 10^{-7}M). $p < 0.05$, compared to NC.



(**a**) (**b**)

Figure 5- Sample metaphases from (a) Positive control, mitomycin-c; (b) Compound **10** (50 µg/mL) treated cultures (arrows show SCE formations).

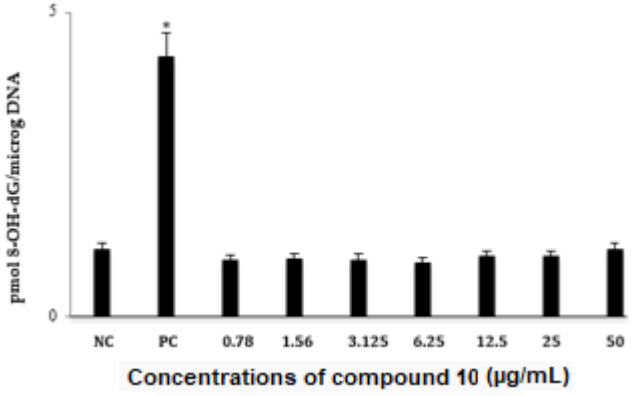


Figure 6– The levels of 8-oxo-2-deoxyguanosine (8-OH-dG) adducts in cultured human blood cells maintained 72 h in the presence of different concentrations of compound **10**.