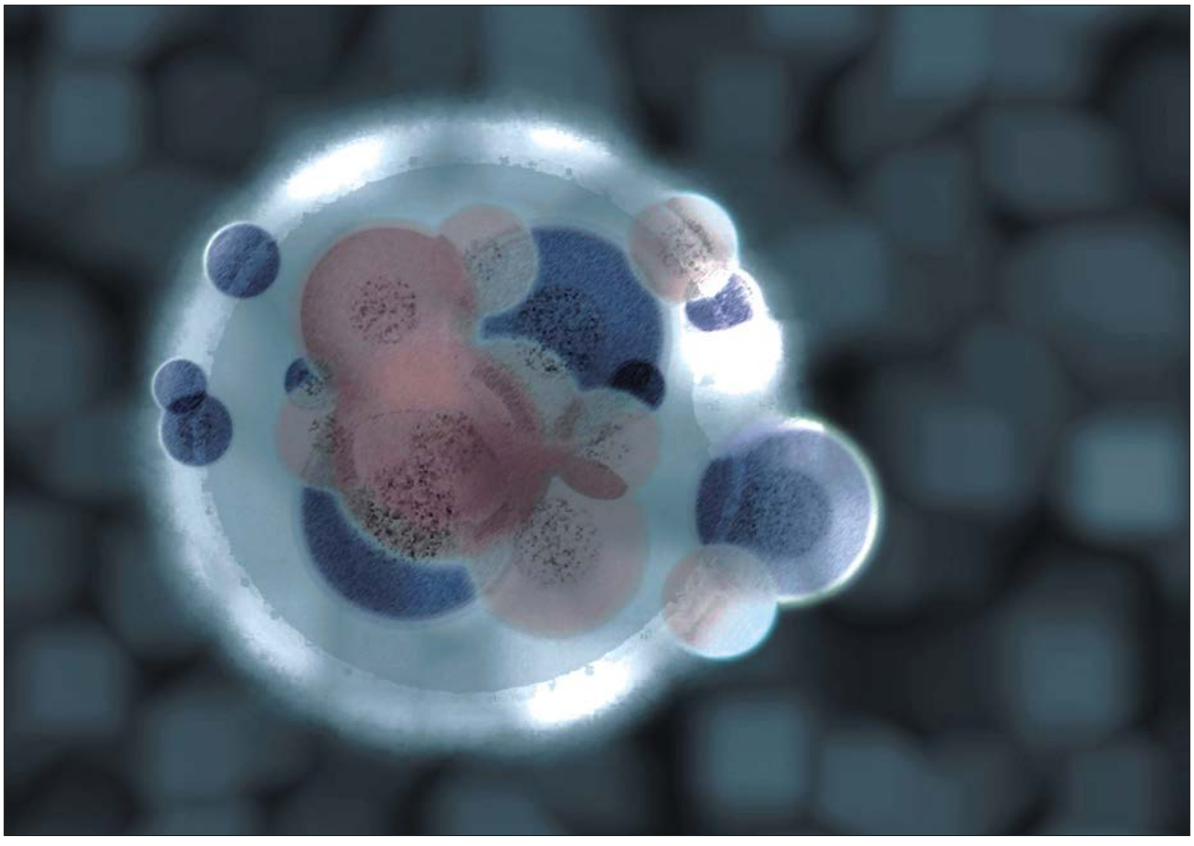

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In vitro Cytotoxic Activity of β-chalcogen-substituted Michael-aldol Type Adducts Against Hela and RKO Cell Lines

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Abstract: There is a lack of biological studies using selenium and tellurium compounds, specially concern with cancer therapy. The aim of this study is to evaluate the cytotoxicity action of nine β-chalcogen-substituted Michael-aldol-type adducts on HeLa and RKO cancer cell lines. The cytotoxic effect was assessed by MTT assay and was performed in HeLa and RKO cell lines cultured in RPMI-1640 medium in (95% O_2+5% CO_2) at 37°C. The IC_{50} values demonstrated that all compounds presented cytotoxic effect in 17-100 μM range. The compounds 1 and 5 presented cytotoxic effect in 17 -40 μM range to HeLa cells. In RKO cells compounds 2-5, 7 and 8 presented cytotoxicity between 46 -58 μM. Compound 1 presented cytotoxicity for HeLa cells similar to those found to etoposide (11.35±2.73 μM; $p>0.05$) and none of the compounds presented this similarity for RKO cells. It is important to notice that the compounds presented cell line selectivity: compound 1 to HeLa and compounds 7 and 8 to RKO cells. The RKO cells were more sensitivity to tellurites, which were not effective to HeLa cells. In conclusion, the compound 1 presented promissory anticancer potential and the cytotoxic specificity of tellurides for RKO cells demonstrated that there is an important biological role based on this chemical element.

Key words: Cancer, chalcogens, selenium, tellurium, michael-aldol

INTRODUCTION

Cancer is one of the highest impacting diseases worldwide because is a debilitating infirmity with significant morbidity and mortality taxes. According to recent studies, it is estimated a continuous increasing on the new cancer cases ever year (Bray *et al*., 2012).

The current known therapies of some types of cancer are based on radio- and chemotherapies and although in many cases, the patients have their health reestablished, the treatment is very painful since their immunological system is severely compromised, because these procedures are not cells selective. This is one of the most critical periods of the treatment because the patient becomes very vulnerable to other diseases. On the other hand, the search for new treatments based on drugs that can act only on the tumor cells, or at least more selectively, preserving intact the health cells, is a challenging field of investigation and many efforts have been directed towards the evaluation of synthetic compounds for this end (Mann, 2002; Neidle and Thurston, 2005).

In the last decades, a myriad of natural and synthetic compounds were screened out for this purpose and with the advent of the medicinal chemistry was demonstrated that the synthetic alternative is so far the most promissory, specially because thousand of new compounds can be prepared in a short period of time for initial screening studies (Neidle and Thurston, 2005).

Beside there is no known evidence of some biological function involving tellurium, in the early 1950s, its counterpart, selenium was identified as a bio-essential element being vital for several organisms, including mammals (Navarro-Alarcon and Lopez-Martinez, 2000).

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Selenocysteine, 21th natural amino acid, is present in the mammalian anti-oxidant glutathione peroxidase, iodothyronine deiodinase, playing role for the conversion of thyroid hormones and in thioredoxin reductase, which is involved in the DNA synthesis (Bhabak and Mugesh, 2010; Doering *et al*., 2010; Antony and Bayse, 2011; Mobli *et al*., 2011; Nascimento *et al*., 2012; Nissan *et al*., 2012).

Probably because the above-mentioned biological roles of selenium-based naturally occurring compounds, many efforts have been devoted on the investigation of synthetic selenium compounds for therapeutic studies. Firstly synthesized in 1924, Ebselen shows to be capable to catalyze the inactivation of hydroperoxides by reaction with thiols. Since this pioneering report, many other biological investigations with organoselenides have been conduced (Bijian *et al*., 2012; Font *et al*., 2012; Hudson *et al*., 2012). Just as few examples of the selenium role on this context, we mention the exert action anti-proliferative and proapoptotic of several cancer cell types and also to inhibit the activity of drug resistance mechanisms to potentiate chemotherapy and radiotherapy treatment efficacy (Madhunapantula *et al*., 2008); angiogenesis in mammary cancer cells (Jiang *et al*., 1999); angiogenic switch regulation caused by selenium-specific inhibition of MMP-2 and VEGF expression (Jiang *et al*., 2000). Other important mechanism for anticancer effect of selenium compounds is the modulation of p53 protein (Smith *et al*., 2004; Fischer *et al*., 2007).

Contrary to the selenium, oxygen and sulfur, the counterparts of tellurium on the periodic table, much less biological investigation are known for compounds of this element. Tellurium based-compounds can occurs in many forms including inorganic as well as organic. This structural flexibility could be very interesting for drug development in biological screening studies and many of the few organotellurides investigated to date presented expressive biological activity like as inhibition of proteins and enzymes and induction of apoptosis in certain cancer cells (Ba *et al*., 2010; Vij and Hardej, 2012). Several works describe the pronounced increase of the antioxidant activity of diaryltellurides compared to the selenium analogs. It is known that antioxidant compounds plays an important role in targeting therapeutic interventions in many diseases. Several p-substituted diaryltellurides demonstrated potent glutathione peroxidase-like activity. Just to compare the relevance of this fact, one of these compounds (bis a-aminophenyl) telluride, demonstrated until 900% of the catalytic activity of Ebselen for the glutathione-dependent reduction of hydrogen peroxide and other commonly used oxidants (Andersson *et al*., 1993).

It was demonstrated that the treatment consisting of a combination of melphalan and AS101 resulted in synergistic *in vitro* inhibition on growth, reduction of IgG_{2b} secretion, G_2/M phase growth arrest, apoptotic cell death and reduction of basal adhesion of MM cells (Hayun *et al*., 2009). As a result of this study it was observed that the associate use of these compounds *in vivo* resulted in modest survival improvement of myeloma-bearing mice and in reduced IgG_{2b} and VEGF serum levels. On the best of our knowledge, AS101 is the only telluride under phase I/II clinical studies for cancer treatment.

In the last years we have dedicated attention on the development of synthetic methodologies on the preparation of selenium and tellurium-based organic compounds as well as their application as precursors in the natural product synthesis (Comasseto and Dos Santos, 2008; Gay *et al*., 2010; Princival *et al*., 2010; Ferrarini *et al*., 2012; Takata *et al*., 2012). Recently we reported a procedure demonstrating a versatility of organometallic selenolates for the promotion of Michael-aldol-type and Morita-Baylis-Hillman reaction, allowing the preparation of a quite diversified group of derivatives (Gariani *et al*., 2008; Sousa and Dos Santos, 2012; Sousa *et al*., 2012).

Herein, we present the cytotoxicity action of nine β-chalcogen-substituted Michael-aldol-type adducts on HeLa and RKO cell lines.

MATERIALS AND METHODS

Cell culture and treatment: HeLa (human cervix carcinoma) and RKO (colon carcinoma) cells were cultured in RPMI medium (Sigma) supplemented with 10% Fetal Bovine Serum (FBS) (Hyclone), streptomycin (60 mg mL^{-1}) and penicillin (100 mg mL^{-1}) in a humidified atmosphere of 5% CO_2. Cells were seeded at 1×10^5 cells/cm^2. Culture medium was changed every 48 h to avoid nutrient depletion.

Cytotoxicity assay: The cytotoxicity effect was assessed using a tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide-supplied from Sigma®, St. Louis, MO, USA) colorimetric method. Briefly, the cells were plated in 96-well plates (1×10^5 cells/well) and incubated for 24 h at 37°C in humid atmosphere with 5% CO_2 to adhesion. After this period, the wells were washed with culture medium and incubated with the compounds at different concentrations. After the incubation, the plates were treated with MTT. The reading was performed in microplate reader Spectramax M5e (Molecular Devices, Sunnyvale, CA, USA) at 550 nm. Cytotoxicity was scored as the percentage reduction in absorbance versus untreated control cultures. All experiments were performed in triplicate. The results were expressed as the mean of IC_{50} (the lethal drug concentration that reduced cell viability to 50%) and were calculated used software Origin® 8.0.

Statistical analyses: Graphics and statistical analyzes were done using the software GraphPad Prism® 5. Were performed at least three independent assays of cell viability and in triplicate for each cell line. One-way analysis of Variance (ANOVA) using Dunnett post test were used. Statistical analyzes were performed considering 95% significance level and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

The cytotoxicity assays (MTT) with the compounds were performed in two different cell lines (HeLa and RKO) to verify the possible anticancer effect of these substances. Etoposide (a classic antitumor drug) was used as positive control. The cell viabilities after the treatment with the compounds were demonstrated in Fig. 1. All compounds showed a dose-dependent effect

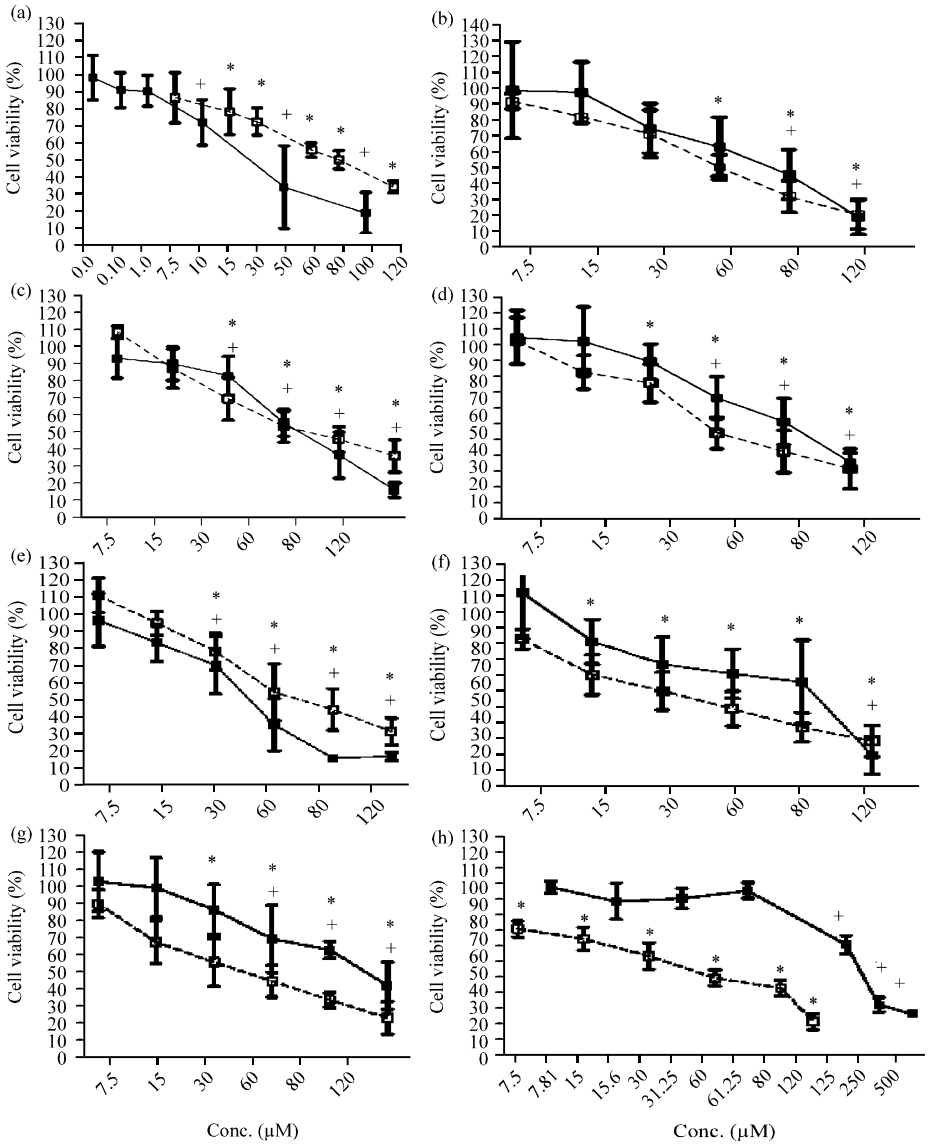
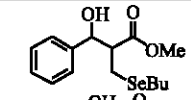
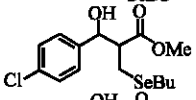
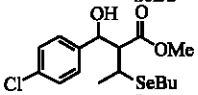
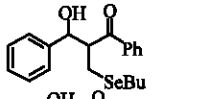
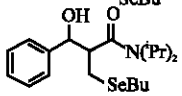
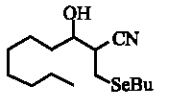
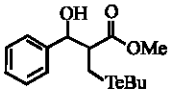
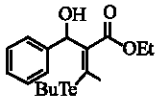


Fig. 1(a-h): Cytotoxic effect of selenides and tellurides for Hela and RKO cell lines. (a) Compound 1, (b) Compound 2, (c) Compound 3, (d) Compound 4, (e) Compound 5, (f) Compound 6, (g) Compound 7, (h) Compound 8, Selenides (1-6) and tellurides (7 and 8) were used to treated HeLa (black square) or RKO (open square) cell lines. Data are Mean±SE (n = 3). Asterisks for RKO and cross for HeLa cells mean that results are significantly different from controls, as evaluated by ANOVA following multiple comparisons with Dunnett test ($p<0.05$)

Table 1: *In-vitro* cytotoxic activity of β-butyl-seleno and -telluro michael-aldol-type adducts

Compound	Structure	IC_{50} (μM)±S.D[a] Hella	RKO
1		17.31±4.95*+	71.82±4./61
2		62.30±19.87	54.72±9.88
3		53.61±8.99	58.69±5.74
4		85.51±13.35	54.39 ±8.18
5		30.89±7.47	48.85±7.01
6		59.52±8.34	76.24±17.05
7		108.43±19.38	46.80±14.84*
8		164.22±27.06	49.47±10.47*
Etoposide		11.35±2.73	3.99±1.46

[a]Values are average±Standard deviation, *Compared to other cell ($p<0.05$), +Compared to Etoposide effect ($p>0.05$)

and compound 1 demonstrated to be more cytotoxic, with starting decrease cell viability with 10 μM for HeLa cells and 15 μM for RKO. Compounds 7 and 8 were more cytotoxic for RKO than HeLa cell lines.

The IC_{50} values demonstrated that all compounds presented cytotoxic effect in 17-100 μM range. The compounds 1 and 5 presented cytotoxic effect in 17-40 μM range to HeLa cells. In RKO cells compounds 2-5, 7 and 8 presented cytotoxicity between 46-58 μM (Table 1). Compound 1 presented cytotoxicity for HeLa cells similar to those found to etoposide (11.35±2.73 μM; $p>0.05$) and none of the compounds presented this similarity for RKO cells. It is important to notice that the compounds presented cell line selectivity: compound 1 to HeLa and compounds 7 and 8 to RKO cells.

It is important co-relate the structure of the compounds with their biological effect, because this comparison can be valuable to propose new lead compounds with better cytotoxic potential. It was observed a difference on the cytotoxic effect of compounds 1 and 7 (chalcogen homologue compounds) suggesting that the chalcogen atom is the key responsible to the cytotoxicity.

The RKO cells were more sensitivity to tellurites, which were not effective to HeLa cells. HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences, a low expression of p53 and a normal expression of pRB (retinoblastoma suppressor) (Scheffner *et al*., 1991). RKO cells, on the other hand, are poorly differentiated by colon carcinoma that contain wild-type expression of p53 and it can be used as the control cell line for investigating the effects of p53 (Brattain *et al*., 1984; Smith *et al*., 1995).

The p53 present the capacity to modulate several cellular processes including apoptosis, cell cycle arrest and DNA repair (Hofseth *et al*., 2004). The p53 mutations can modified expression and protein structure, causing loss or change of p53 binding activity to its downstream targets leading to malignant cellular transformation (Oren, 2003). Based on p53's role in cancer development, the p53 become a critical role for new drugs and several strategies for treating cancer was adopted by enhancing function of wild-type p53 or increasing p53 stability (Bai and Zhu, 2006; Lane *et al*., 2010). The cytotoxic effect of tellurides to RKO cells instead of HeLa cells can be attributed to the possible effect of p53 protein and

because of the difference of expression, only RKO cells were sensitive to the telluride effect. However, there are some reports showing the modulation of p53 pathway as a mechanism of anticancer effect of selenium compounds (Smith *et al*., 2004; Fischer *et al*., 2007). More experiments are important to understand which mechanisms are affected by tellurides and selenides in these cells.

CONCLUSION

We demonstrate the cytotoxic effect of β-butyl-seleno and-teluro-substituted Michael-aldol type adducts for Hela and RKO cell lines and the compound 1 presented promissory anticancer potential. The cytotoxic specificity of tellurides for RKO cells demonstrated that there is an important biological role based on this chemical element.

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