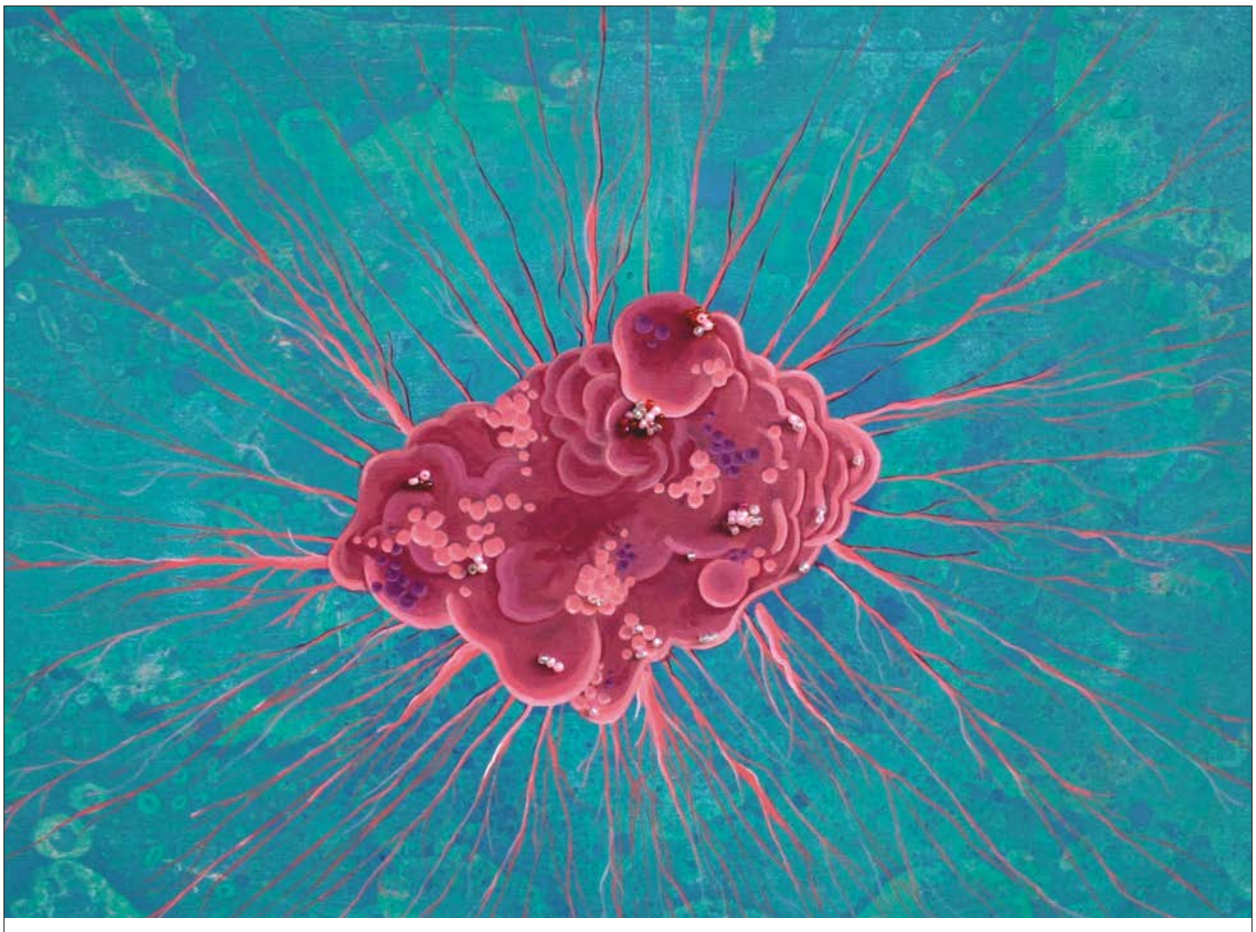

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Anti-tumor Activity of New Quinoline Derivatives in Human Breast Cancer T47D Cells

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Abstract: New cytotoxic quinoline derivatives were designed, synthesized and evaluated *in vitro* as anti-tumor agents in comparison to available drugs including Adriamycin (ADR), Vincristin (VCR), Etoposide (VP16) and Tamoxifen (TAM). Human breast cancer T47D cells were cultured in RPMI 1640 complete culture medium and exposed for 48 h to different concentrations of newly synthesized quinoline derivatives [SRA-HX-(1-3) and SRA-BQ] and also to ADR, VCR, VP16 and TAM. A dose-dependent decrease in cell proliferation was observed following exposure to almost all synthesized quinolines. The highest cytotoxicity was seen at $1x10^{-4}$M concentration of SRA-HX-3 that was near to growth inhibitory effect of ADR ($1x10^{-6}$M) and significantly ($p<0.002$) greater than VCR, VP16 and TAM (each at $1x10^{-6}$M). The other 3 compounds ($1x10^{-4}$M) had similar activity to VCR that was less than ADR and significantly ($p<0.002$) greater than VP16 and TAM. Therefore, new cytotoxic quinolines are potentially good candidates for further investigation as anti-tumor compounds.

Key words: Breast cancer, anti-tumor, azoles, quinoline derivatives, adriamycin

Introduction

The incidence and mortality of breast cancer among women worldwide have become one of the most important medical issues. With progress in understanding of the pathobiology of breast cancer, diagnosis and treatments have been significantly improved (Dos Santos Silva *et al*., 2003; Engel *et al*., 2003; Piccart *et al*., 2003). Unfortunately, development of resistance to chemotherapeutic agents is a common obstacle in the treatment of different types of cancers including breast cancer (Li *et al*., 2001; Arancia *et al*., 2001). Several important drugs including Adriamycin (ADR), Vincristin (VCR) and Etoposide (VP16) with different structures and

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mechanisms of anti-tumor activities fail to be effective due to drug resistance (Whelan *et al*., 1992; Uchiyama-Kokubu *et al*., 2001; Cre *et al*., 2002; Faneyte *et al*., 2001). Adriamycin, an anthracycline anti-tumor drug, is clinically active against many human malignancies including breast cancer (Son *et al*., 1998; Callies *et al*., 2003; Larsen and Skladanowski, 1998). Several mechanisms explain the antitumor activity of ADR including DNA intercalation, inhibition of topoisomerase II (TOPO II), interaction with membrane and generation of oxygen free radicals (Gewirtz, 1999; Dunkern *et al*., 2003). Vincristin is a vinca alkaloid anti-tumor agent, which exerts its effect by binding to tubulin and therefore, inhibiting microtubule formation during mitosis (Whelan *et al*., 1992). Etoposide is a derivative of podophyllotoxin, which inhibits TOPO II and is believed to cause breakdown of DNA (Schneider *et al*., 1994). Tamoxifen (TAM) is a non-steroidal anti-estrogen that is widely used for preventing and treatment of breast tumors (Fouladdel *et al*., 2005). Due to failure of anti-tumor drugs to exert their effects in certain cases of cancers, looking for new chemotherapeutic agents with synthetic or natural origins is one of the hot topics in cancer research laboratories. Therefore, in the present study we evaluated the anticancer effects of several newly designed and synthesized quinoline derivatives to be compared with available drugs.

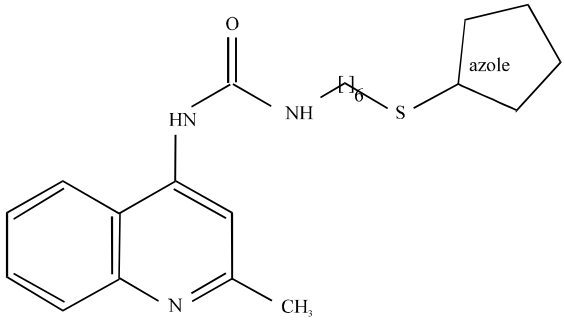
Materials and Methods

Cell line and Culture Conditions

The human breast cancer T47D cell line (ATCC HTB-133, USA) was obtained from Pasteur Institute Cell Bank of IRAN (Tehran, IRAN). Cells were maintained in RPMI-1640 (Gibco, UK) culture medium supplemented with 10% fetal bovine serum (Gibco, UK) and 100 U mL^{-1} of penicillin and 100 ng mL^{-1} of streptomycin (Gibco, UK) at 37°C in 5% CO_2 incubator. All reagents and chemicals used in this experiment were of cell culture or molecular biology grade, purchased from different international sources.

Chemical Structure of Synthesized Compounds

A series of azolylalkylquinolines have been previously reported as potent and selective cytotoxic agents *in vitro* (Abel *et al*., 1996a, b, c). In the continuation of development of cytotoxic quinolines, 3 novel azolylthioalkyl-ureidoquinoline derivatives (Fig. 1) named SRA-HX-(1-3) and also a biuret



SRA-HX(1-3)

Fig. 1: Chemical structure of azole quinolines. New cytotoxic quinoline derivatives with different azole groups. SRA-HX-1: benzothiazole, SRA- HX-2: tetrazole, SRA-HX-3: 1,2,4-triazole

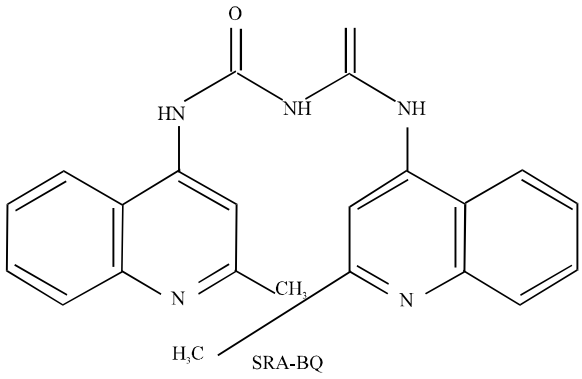


Fig. 2: Chemical structure of Biuret quinoline. New cytotoxic quinoline derivative with biuret group named SRA-BQ

quinoline derivative named SRA-BQ (Fig. 2) were designed and synthesized to be evaluated as new anti-tumor compounds in comparison to available anti-tumor drugs.

Cytotoxicity Assay

The MTT (3-[4, 5-dimethylthiazol - 2 - yl]-2, 5-diphenyl tetrazolium bromide) based assay was performed by seeding 20,000 cells per 180 µL RPMI complete culture medium in each well of 96-well culture plates. The day after seeding, culture medium was changed with medium containing standard anti-tumor drugs including ADR, VCR, VP16 and TAM as well as different concentrations of newly synthesized compounds [SRA- HX (1-3) and SRA-BQ] and RPMI control (no drug). Cells were then incubated at 37°C in 5% CO_2 incubator for 48 h. Then, 25 µL of MTT solution (4 mg mL^{-1}) were added to each well and further incubated at 37°C for 3 h. At the end of incubation, formazan crystals were dissolved in 100 µL of DMSO and plates were read in a plate reader (TECAN, Austria) at 540 nm. This experiment was performed in triplicate determination each time.

Statistical Analysis

SPSS 11 was used to perform statistical analysis of data. The ANOVA test with Dunnet post hoc analysis was used to examine the differences among treatments. Mean differences with P values less than 0.05 were considered to be statistically significant.

Results and Discussion

Following exposure of T47D cells to standard and synthesized compounds, cell growth was compared to RPMI control to determine the cytotoxicity of each compound *in vitro*. The cytotoxicity of synthesized compounds was found to be dose-dependent with exception of compound SRA-HX-2 (Fig. 3-6). The anti-tumor effect of compound SRA-HX-3 (Fig. 5) at $1x10^{-4}$M concentration was near to ADR ($1x10^{-6}$M) and significantly ($p<0.002$) greater than VCR, VP16 and TAM, each at $1x10^{-6}$M concentration. The other 3 compounds (at $1x10^{-4}$M) had almost similar activity to VCR that was less than ADR and significantly ($p<0.002$) greater than VP16 and TAM (Fig. 3, 4 and 6).

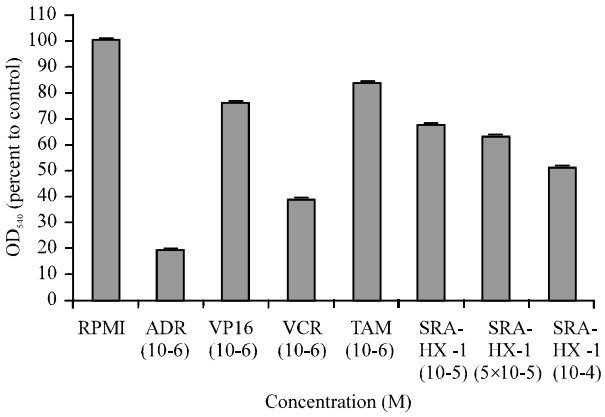


Fig. 3: Anti-tumor activity of SRA-HX-1. The human breast cancer T47D cells were seeded in 96-well culture plates and exposed to standard anticancer drugs, different concentrations of SRA-HX-1 and RPMI control (no drug) for 48 h in CO_2 incubator at 37°C. Cytoxicity was then determined using MTT assay as described in methods. Data are mean±SD of triplicate determination in each experiment (N = 3)

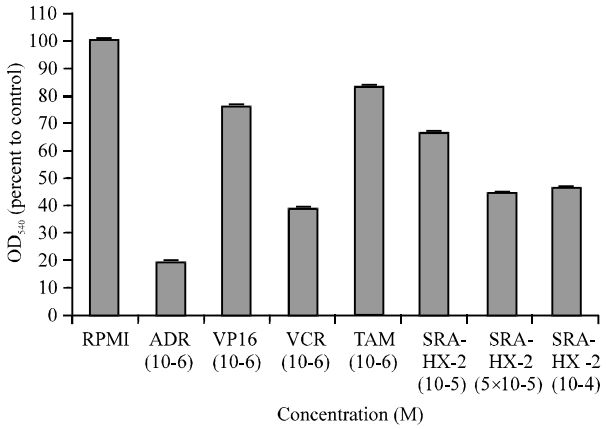


Fig. 4: Anti-tumor activity of SRA-HX-2. The human breast cancer T47D cells were seeded in 96-well culture plates and exposed to standard anticancer drugs, different concentrations of SRA-HX-2 and RPMI control (no drug) for 48 h in CO_2 incubator at 37°C. Cytoxicity was then determined using MTT assay as described in methods. Data are mean±SD of triplicate determination in each experiment (N = 3)

Despite cases of failure in treatment of certain cancers, chemotherapy remains one of the most important and effective ways of cancer therapy. Therefore, to improve chance of tumor eradication, particularly in case of drug resistance of tumor cells, it is necessary to evaluate new compounds with synthetic or natural origins. In this study, we evaluated the anti-tumor effects of 4 newly synthesized compounds that were quinoline derivatives. In this study, all compounds were cytotoxic *in vitro* with

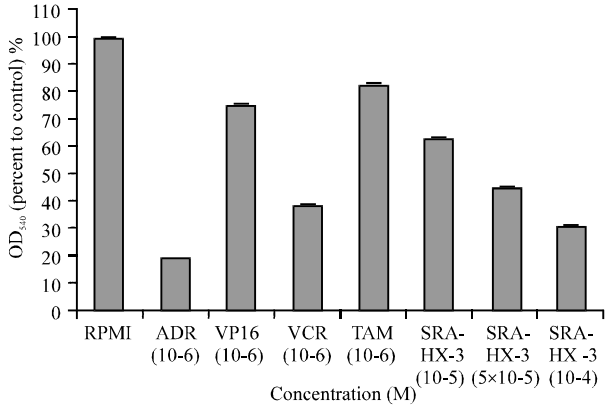


Fig. 5: Anti-tumor activity of SRA-HX-3. The human breast cancer T47D cells were seeded in 96-well culture plates and exposed to standard anticancer drugs, different concentrations of SRA-HX-3 and RPMI control (no drug) for 48 h in CO_2 incubator at 37°C. Cytoxicity was then determined using MTT assay as described in methods. Data are mean ± SD of triplicate determination in each experiment (N = 3)

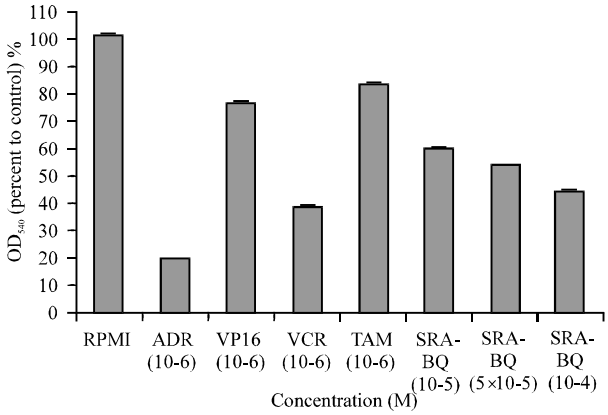


Fig. 6: Anti-tumor activity of SRA-BQ. The human breast cancer T47D cells were seeded in 96-well culture plates and exposed to standard anticancer drugs, different concentrations of SRA-BQ and RPMI control (no drug) for 48 h in CO_2 incubator at 37°C. Cytoxicity was then determined using MTT assay as described in methods. Data are mean±SD of triplicate determination in each experiment (N = 3)

less potency than ADR ($1x10^{-6}$M). The most cytotoxic compound in the present study was an azole derivative of quinoline named SRA-HX-3. Other quinoline derivatives have also previously shown to be potent cytotoxic compounds *in vitro* (Abel *et al*., 1996a, b, c). Among synthesized quinoline derivatives by other investigators, compounds 9q (Abel *et al*., 1996a), 7g (Abel *et al*., 1996b) and 27 (Abel *et al*., 1996c) were more cytotoxic on KB cells (human nasopharyngeal carcinoma) and L1210 cells (Murine lymphocytic leukemia) in comparison to ADR and other synthesized quinolines in each report. Despite cytotoxic activity of newly synthesized quinolines *in vitro*, further

investigation is required to determine the exact mechanism of action, adverse effects and pharmacokinetic parameters of these derivatives for better comparison with available anti-tumor drugs.

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