

Cytotoxicity of some 1-(2,4-dihydroxyphenyl)-3-(4-phenylpiperidin-1-yl)prop-2-en-1-one derivatives using MTT assay

Farzaneh Fesahat¹, Mehdi Khoshneviszadeh², Alireza Foroumadi³, Alireza Vahidi¹, Maryam Fereidounpour¹, Amirhossein Sakhteman^{2*}

¹Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

²Department of Medicinal Chemistry, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

³Department of Medicinal Chemistry, School of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Background: Some N-substituted piperidine structures were synthesized and evaluated for cytotoxicity.

Aim and significance: The N-substituted piperidine with propene substructure could be considered as a lead structure for further studies of structure activity relationship to develop more potent compounds in future.

Materials and methods: The compounds were evaluated against four different cell lines using the

standard MTT assay method and Doxorubicin was used as the reference drug.

Results and discussion: The IC₅₀ values were determined by constructing dose-response curves and revealed that three of the synthesized compounds were active against breast cancer cell lines. Compound 6a bearing hydroxyl at para position of piperidine ring was the most active compound within this series.

Keywords: Piperidine, Cytotoxicity, Breast cancer

1. Introduction

Cancer, as a broad group of various diseases, is normally expressed by unregulated cell growth. In many types of cancer, uncontrollable cell division could result in the formation of malignant tumors which attack the adjacent parts of the body. In 2007, cancer caused about 13% of all human deaths around the world (7.9 million)(1). There are over 200 different known human cancers (1). Ovarian cancer is a common neoplasm in Western countries and the leading cause of death in women with gynecological malignancies. The heterogeneity of ovarian cancer forces the researcher to find new strategies and targets to individualize the therapy of this common disease (1). Burkitt lymphoma is a high-grade, rapidly-growing and aggressive B-cell non-Hodgkin's lymphoma, with three recognized forms: those endemic to Africa, sporadic forms, and those associated with immunodeficiency states (2). Human foreskin fibroblast (HFFF2) is a normal fibroblast like stem cell obtained from human fore-

skin fibroblast and is used as a predictive model. The most usual treatments for cancer are based on chemotherapy, radiation therapy and surgery. Radiotherapy consists of a vital element in the treatment of breast cancer but has limiting factors for a successful treatment such as relative side effects and different radioactive responses (3).

Chemotherapy is therefore a noninvasive method wherein cytotoxic agents are used in the treatment of the disease. Doxorubicin as a successful example of chemical agents was used to treat cancer since 1960s, and its clinical pharmacology was well established (Figure 1) (4). It is approved worldwide for the treatment of leukemia, bone cancer, multiple myeloma and ovarian carcinoma and is considered as the most effective drug in the treatment of breast cancer (5,6). Due to multi drug resistance for chemotherapy agents, demand for newer agents has been a great challenge in pharmaceutical sciences (7). Different derivatives bearing piperidine substructure were previously reported as anti cancer agents (8,9). In a recent study insertion of piperidine ring into benzofuran scaffold has led to a dramatic increase in the activity of the synthesized compounds as inhibitors of mTOR (mamma-

Corresponding Author: Amirhossein Sakhteman, Department of Medicinal Chemistry, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

Tel/Fax: +98 711-242-4128

E-mail: asakhteman@sums.ac.ir

lian target of rapamycin) signaling, a newly discovered target in oncology (Figure 2) (10). In order to extend a reasonable structure activity relationship pattern for the piperidine structures and to find out a proper lead structure for further studies, some piperidine structures were synthesized and evaluated for anti cancer activity in this study. The selection of analogues for evaluation was in such a way that a whole overview of the chemical space could be obtained with the least time and material consumption. The cytotoxicity of the compounds was determined using MTT assay (11).

2. Materials and Methods

2.1. Synthesis of the target compounds (6a-d)

Synthesis and purification of the target compounds, 3-(Piperidin-1-yl)-1-(4-substituted phenyl)prop-2-en-1-one derivatives (6a-d), has been carried out according to the previously described procedure (Scheme 1) (12). As depicted in Scheme 1, annulation of chromanone ring preceded by primary acylation of resorcinol yielded compound 3. Subsequently, the hydroxyl moiety was alkylated and a further mono bromination reaction was conducted. After then, the compound was reacted with piperidine to afford the final compounds according to the previously described rearrangement (12).

2.2. Cytotoxicity activity

2.2.1. Materials

Doxorubicin, RPMI-1460 and L-glutamine were purchased from Sigma, and Antibiotic/Antimicotic Solution and fetal bovine serum were purchased from Gibco (Grand Island, NY). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Roche (Applied Science, Indianapolis, IN).

2.2.2. Cell lines

Ovarian cancer (OVCAR3), Human Burkitt's Lymphoma (CA46), Breast cancer (T47d) cell lines and human foreskin fibroblast (HFFF2) were purchased from Pasteur Institute of Iran. The cells were cultured in polystyrene plates enriched with Dulbecco's Modified Eagle Medium (DMEM) and RPMI 1640 medium (Gibco BRL), supplemented with 10% fetal bovine serum, 100 IU/ml penicillin (Sigma) and 100 mg/ml streptomycin (Sigma), and incubated at 37 °C in a humid atmosphere with 5% CO₂. All lineages were maintained in the logarithmic phase. The media were changed twice weekly and regular examinations were performed. Evaluation of the cytotoxic effect against all lineages were inoculated at 10,000 cells per well. The plates were preincubated for 24 h at 37 °C to allow adaptation of cells prior to the addition of the test compounds. Freshly prepared solutions of the different compounds were tested at 10 μM. Subsequently, the plates were inoculated for 24 h in an atmosphere of 5% CO₂ and 95% relative humidity. Control groups

included treatment with 0.1% DMSO (negative control) and 10 μM of doxorubicin (positive control). All cells were monitored under optical microscopy (400×) before addition of MTT solution to evaluate the morphological changes in vitro after treatment of different concentrations of each compound. Cell viability was estimated by measuring the rate of mitochondrial reduction of MTT. All substances were dissolved in dimethyl sulfoxide (DMSO) prior to dilution. Compounds that inhibited the proliferation in more than 50% were selected for determination of the half maximal inhibitory concentration (IC₅₀). IC₅₀ values were determined over a range of concentrations (10-100 μM). All compounds were tested in triplicate, in three independent experiments (13).

2.3. In vitro cell viability assay (MTT assay)

The MTT [3-(4, 5-dimethyl-2-thiazolyl) -2, 5-diphenyl -2H- tetrazolium bromide] assay is a standard colorimetric assay, in which mitochondrial activity is measured by splitting tetrazolium salts with mitochondrial dehydrogenases in viable cells only. Briefly, after 4 h of the end of incubation of cells with different compounds, 20 μL of MTT solution (2.5 mg mL⁻¹ in phosphate-buffered saline) was added to each well, the supernatant was removed and 200 μL of 0.04 M HCl in isopropyl alcohol was added to dissolve the formazan crystals. The optical densities (OD) were evaluated at 570 nm using a Rayleigh spectrophotometer. Controls included drug-containing medium (background) and drug-free complete medium. Drug-free complete medium was used as control (blank) and was treated in the same way as the drug-containing media. Results were expressed as percentage of cell proliferation, comparing with 0.1% DMSO control and were calculated as follows: viability (%)=(mean OD treated - mean OD background)/(mean OD untreated cultured, i.e. 0.1% DMSO - mean OD blank wells) × 100. Interactions of compounds and media were estimated on the basis of the variations between drug-containing medium

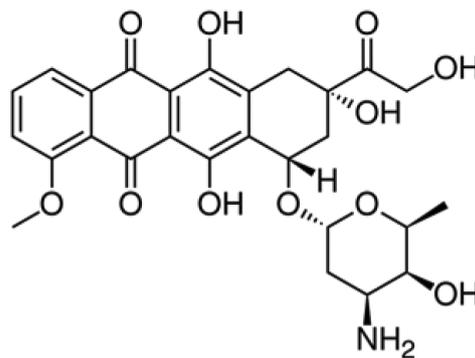
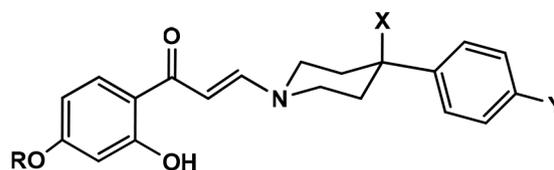


Figure 1. The structure of the chemotherapy agent, Doxorubicin

to propene linker and result in compounds with narrow spectrum (6c and 6d). Despite a narrow spectrum for compound 6c, it was more active than doxorubicin against T47d cell line. The cytotoxicity of the studied compounds was also evaluated in normal cells. The human HFF cell line was used as the predictive model. For this purpose (TI) therapeutic index was calculated as $TI = IC_{50} (HFF)/IC_{50} (tumor\ cell)$. According to Table 1, although compound 6a presented the highest cytotoxicity against the investigated tumor cell lines, it could be considered as the best antitumor drug candidate due to its higher therapeutic indexes. The TI values for compound 6a were 1.8, 2.95 and 3.17 for the cell lines OVCAR3, CA46 and T47d, respectively. In contrast, the obtained TI value for doxorubicin indicates its low toxicity of normal cells against OVCAR3 cells ($TI=3.23$) while compound 6a showed lower toxicity to normal cells in case of T47d ($TI=3.17$) and CA46 ($TI=2.95$) cell lines. The morphological changes in all cell lines especially adherent cells such as HFF, OVCAR3 and T47d were clear in low concentrations ($25\mu M$) for all the synthesized structures. This might indicate the chemical contamination for lineage cells, and could alter the normal biological behavior in vitro. Ultra structure, gene expression profiles and in vivo study of these compounds might be required to show other features of their effects on live normal and tumor cell lines in order to describe cell and molecular mechanisms of their functions. The mechanism of activity for the flat anti cytotoxic compounds could be through intercalation with DNA(17). Since these compounds are unsaturated carbonyl compounds conjugated with phenyl ring, it was inferred that the plausible mecha-



nism for their activity is DNA intercalation. To deal with this hypothesis, the most active compound, 6a, was subjected to docking simulation study with DNA. The best docking pose in terms of ΔG is depicted in Figure 3. It seems that the flat part of the molecule is well fitted between C-G base pairs of DNA with a negative binding energy (-6.8 kcal) suggesting an intercalating mechanism for this compound.

4. Conclusion

Four N-substituted piperidine structures were synthesized and evaluated for anti cancer activity against four cell lines. The experiments were designed so that some minor but principal modifications of the substituents could be studied with the few synthesized structures.

It was shown that all compounds except 6d lacking hydroxyl at para position of piperidine ring were active agents against T47d with the order of $6a > 6c > 6b$ based on their IC_{50} values. An important issue with the structure activity relationship of this series of compounds was the presence of OH at para position of piperidine ring. Other outstanding modification was ortho modification in the phenyl group attached to propene

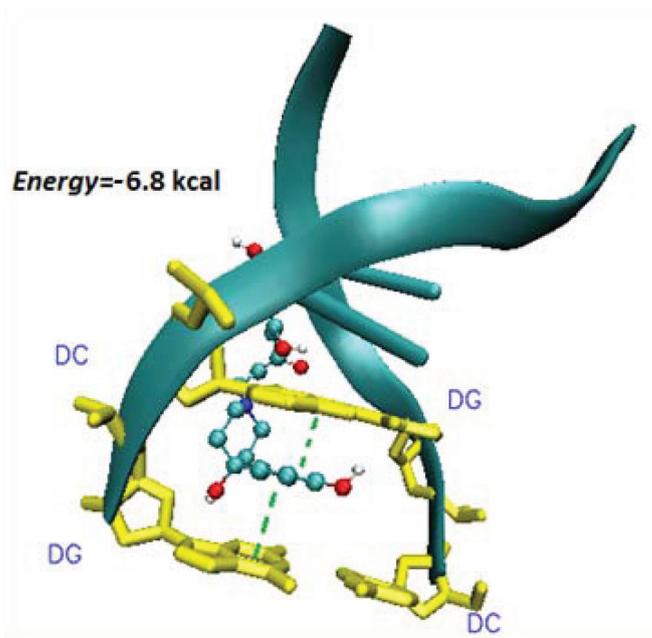


Figure 3. Intercalation of compound 6a with DNA at C-G base pair site using docking simulation.

Table 1. Cytotoxicity (IC₅₀ values) of compounds 6a-d against different cell lines

Compounds		IC ₅₀ (μM) 95% Confidence interval in parenthesis			
		OVCAR3	CA46	HFF	T47d
6a	R=H	89.28	55.64*	>100	51.90*
	X=OH	(46.17- 94.74)	(69.32- 180.61)		(7.36- 89.17)
	Y=OH	TI**=1.8	TI=2.95		TI=3.17
6b	R=H	80.92	>100	96.81	90.19
	X=OH	(49.43- 114.27)		(68.72-124.89)	(54.34-180.9)
	Y=Cl	TI=1.96			TI=1.07
6c	R=CH ₃	88.12	>100	>100	59.85*
	X=OH	(24.07-200.31)			(33.03 -152.73)
	Y=Br	TI=1.15			TI=1.7
6d	R=CH ₃	>100	>100	>100	>100
	X=H				
	Y=OH				
Doxorubicin		28.5845 (16.66-73.83) TI=3.23	63.08 (50.88-75.27) TI=1.46	92.88 (64.12-121.64)	84.78 (43.61-125.94) TI=1.09

* P-value < 0.05 in comparison with the positive control (Doxorubicin); ** TI represents Therapeutic Index

linker at N moiety of the ring. This modification was able to devastate the planarity of the conjugated system. Based on the TI values compound 6a could be proposed as a convenient lead compound for further studies of structure activity relationship against breast cancer cell lines.

Conflict of Interest:

None declared.

5. References

- Ignatov T, Modl S, Thulig M, Weißenborn C, Treeck O, Ortman O, Zenclussen A, Costa, S D, Kalinski T, Ignatov A. GPER-1 acts as a tumor suppressor in ovarian cancer. *J Ovarian Res.* 2013;6:51-61. doi: 10.1186/1757-2215-6-51
- Huang Y, Hu J, Zheng J, Li J, Wei T, Zheng Z, Chen Y. Down-regulation of the PI3K/Akt signaling pathway and induction of apoptosis in CA46 Burkitt lymphoma cells by baicalin. *J Exp Clin.* 2012;31:48 doi: 10.1186/1756-9966-31-48.
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin.* 2011;61:69-90. doi: 10.3322/caac.20107
- Speth PA, van Hoesel QG, Haanen C. Clinical pharmacokinetics of doxorubicin. *Clinical pharmacokinetics.* 1988;15(1):15-31.
- La-Beck NM, Zamboni BA, Gabizon A, Schmeeda H, Amantea M, Gehrig PA, Zamboni W C. Factors affecting the pharmacokinetics of pegylated liposomal doxorubicin in patients. *Cancer Chemother Pharmacol.* 2012;69(1):43-50 .doi: 10.1007/s00280-011-1664-2.
- Aghaee F, Islamian JP, Baradaran B, Mesbahi A, Mohammadzadeh M, Jafarabadi MA. Enhancing the Effects of Low Dose Doxorubicin Treatment by the Radiation in T47D and SKBR3 Breast Cancer Cells. *J Breast Cancer.* 2013;16(2):164-70.
- Hofmann J, Ueberall F, Egle A, Grunicke H. B-859-35, a new drug with anti-tumor activity reverses multi-drug resistance. *Int J Cancer.* 1991;47(6):870-4.
- Poon GK, McCague R, Griggs LJ, Jarman M, Lewis IA. Characterisation of metabolites of 3-ethyl-3-(4-pyridyl)-piperidine-2,6-dione, a potential breast cancer drug. *J Chromatogr.* 1991;572(1-2):143-57.
- Kankala S, Kankala RK, Balaboina R, Thirukovela NS, Vadde R, Vasam CS. Pd-N-heterocyclic carbene catalyzed synthesis of piperidine alkene-alkaloids and their anti-cancer evaluation. *Bioorg med chem lett.* 2014;24(4):1180-3.
- Salome C, Narbonne V, Ribeiro N, Thuad F, Serova M, de Gramont, A. Faivre S, Raymond E, Desaubry L. Benzofuran derivatives as a novel class of inhibitors of mTOR signaling. *Eur J Med Chem.* 2014; 74(3):41-49.
- Sarzaem A, Zare Mirakabadi A, Moradhaseli S, Morovvati H, Lotfi M. Cytotoxic effect of ICD-85 (venom-derived peptides) on HeLa cancer cell line and normal LK cells using MTT Assay. *Arc Iran med.* 2012;15(11):696-701.
- Sakhteman A, Foroumadi A, Sharifzadeh M, Amanlou M, Rayatnia F, Shafiee A. Synthesis and dopaminergic activity of some E-3-(piperidin-1-yl)-1-(4-substituted phenyl)prop-2-en-1-one derivatives. *Bioorg Med Chem.* 2009;17(19):6908-13.
- Ulukaya E, Colakogullari M, Wood EJ. Interference by anti-cancer chemotherapeutic agents in the MTT-tumor chemosensitivity assay. *Chemotherapy.* 2004;50(1):43-50.
- Schuurs AH, Duivenvoorden HJ, van 't Hof MA. [Statistics for dentists (VIII). Student t-test]. *Ned Tijdschr Tandheelkd.* 1993;100(11):495-8.
- Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem.* 2010;31(2):455-61.
- Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. *Journal of molecular graphics.* 1996;14(1):33-8, 27-8.
- Gilad Y, Senderowitz H. Docking studies on DNA intercalators. *J Chem Inf Model.* 2014;54(1):96-107.