Berkala Ilmu Kedokteran
Vol. 39, No. 1, Maret 2007: 1-6

Cytotoxic activity of *N*-alkyl and *N*-benzyl 1,10-phenanthroline derivatives in human cancer cell lines

Eti Nurwening Sholikhah[1], Mustofa[1], Isnatin Miladiyah[2], Ruslin Hadanu[3], Iqmal Tahir[3], Jumina[3], Mahardika Agus Wijayanti[4], Supargiyono[4]

[1]Department of Pharmacology & Toxicology Faculty of Medicine, Gadjah Mada University (GMU), [2]Department of Pharmacology, Faculty of Medicine, Indonesian Islamic University, [3]Department of Chemistry, Faculty of Mathemathics and Natural Sciences, [4]Department of Parasitology, Faculty of Medicine, GMU, Yogyakarta

ABSTRACT

Eti Nurwening Sholikhah[1], Mustofa[1], Isnatin Miladiyah[2], Ruslin Hadanu[3], Iqmal Tahir[3], Jumina[3], Mahardika Agus Wijayanti[4], Supargiyono[4] - ***Cytotoxic activity of N-alkyl and N-benzyl 1,10-phenanthroline derivatives in human cancer cell lines***

Background: In our study on the antiplasmodial activity of 1,10-phenanthroline derivatives, we found some compounds possessing a potential cytotoxic in normal cell line.
Objective: In this study we tested these derivatives in human cancer lines in order to know their *in vitro* anticancer activity.
Materials and methods: Six derivatives of 1,10-phenanthroline, 4 derivatives of *N*-alkyl and 2 derivatives of *N*-benzyl 1,10-phenanthroline were tested on two human cells cancer, myeloma (NS-1) and HeLa cells line. Cytotoxic activity was evaluated by trypan blue exclusion assay and their activity was expressed by the concentration inhibiting 50% of the cell growth (IC_{50}). The IC_{50} of each compound was determined by probit analysis.
Results: The results showed that the IC_{50} values of 1,10-phenanthroline derivatives ranged from 4.68 to 15.63 μM on myeloma cell and from 2.82 to 16.89 μM on HeLa cell. The 4-Bromo-3(2-bromoetyl)-2-metyl-1,10-phenantroline (3) and (1)-*N*-(4-benzyloxy-3-methoxy-benzyl)-1,10-phenanthrolinium chloride (6) with IC_{50} values ranged from 4.68 to 4.72 μM on myeloma cell showed the same ($p>0.05$) cytotoxicity with doxorubicin, with IC_{50} values ranged from 2.82 to 3.08 μM on HeLa cell showed the higher ($p<0.05$) cytotoxicity than doxorubicin. The 4-Chloro-3(2-chloroetyl)-2-metyl-1,10-phenantroline (4) with IC_{50} values 4.77 + 1.58 μM on myeloma cell showed the same cytotoxicity ($p<0.05$) with doxorubicin.
Conclusion: The 4-Bromo-3(2-bromoetyl)-2-metyl-1,10-phenantroline(3) and (1)-*N*-(4-benzyloxy-3-methoxy-benzyl)-1,10-phenanthrolinium chloride (6) revealed potent *in vitro* anticancer activity on both myeloma (NS-1) and HeLa cells lines. The 4-Chloro-3(2-chloroetyl)-2-metyl-1,10-phenantroline (4) revealed potent *in vitro* anticancer activity on myeloma (NS-1) cells line. Further study will be conducted to evaluate *in vivo* anticancer activity on animal cancer model.

Key words: 1,10-phenanthroline, cytotoxic, *in vitro*, myeloma cell, HeLa cell.

Eti Nurwening Sholikhah, Mustofa, Department of Pharmacology & Toxicology, Faculty of Medicine, Gadjah Mada University. Isnatin Miladiyah, Department of Pharmacology, Faculty of Medicine, Indonesian Islamic University, Yogyakarta. Ruslin Hadanu, Iqmal Tahir, Jumina, Department of Chemistry, Faculty of Mathemathics and Natural Sciences. Department of Pharmacology, Faculty of Medicine, Indonesian Islamic University. Jumina, Mahardika Agus Wijayanti[4], Supargiyono Department of Parasitology, Faculty of Medicine, GMU, Yogyakarta

ABSTRAK

Eti Nurwening Sholikhah, Mustofa, Isnatin Miladiyah, Ruslin Hadanu, Iqmal Tahir, Jumina, Mahardika Agus Wijayanti, Supargiyono - ***Aktivitas sitotoksik in vitro derivat N-alkil dan N-benzil 1,10-fenantrolin pada sel kanker manusia.***

Latar belakang. Pada penelitian kami tentang aktivitas antiplasmodium dari derivat 1,10-fenantrolin, ditemukan beberapa senyawa yang mempunyai aktivitas sitotoksik terhadap sel normal.
Tujuan. Pada penelitian ini diuji derivat-derivat senyawa tersebut terhadap sel kanker manusia untuk mengetahui aktivitas antikanker mereka *in vitro*.
Bahan dan cara. Enam derivat 1,10-fenantrolin (4 derivat N-alkil dan 2 derivat N-benzil 1,10-fenantrolin) diuji terhadap 2 macam kanker sel manusia yaitu sel mieloma (NS1) dan Hela. Aktivitas sitotoksik dievaluasi dengan *tryphan blue exclusion assay* dan aktivitasnya dinyatakan sebagai konsentrasi yang menghambat 50% pertumbuhan sel (IC_{50}). Nilai IC_{50} masing-masing senyawa ditentukan dengan analisis probit.
Hasil. Hasil penelitian menunjukkan nilai IC_{50} derivat 1,10-fenantrolin berkisar dari 4,68 sampai 15,63 μM terhadap sel mieloma dan 2,82 – 16,89 μM terhadap sel Hela. Senyawa 4-Bromo-3-(2-bromoetil)-2-metil-1,10-fenantrolin (3) dan (1)-N-(4-benziloksi-3-metoksi-benzil)-1,10-fenantrolin klorida (6) dengan nilai IC_{50} yang berkisar dari 4,68 sampai 4,72 uM terhadap sel mieloma menunjukkan sitotoksitasnya yang sama ($p > 0,05$) dengan doksorubisin; dengan nilai IC_{50} yang berkisar dari 2,82 sampai 3,08 μM terhadap sel Hela menunjukkan sitotoksitas yang lebih tinggi ($p < 0,05$) dibanding doksorubisin. Senyawa 4-kloro-3-(2-kloroetil)-2-metil-1,10-fenantrolin (4) dengan nilai IC_{50} 4,77 + 1,58 terhadap sel mieloma menunjukkan sitotoksisitas yang sama dengan doksorubisin ($p > 0,05$).
Simpulan. Senyawa 4-Bromo-3-(2-bromoetil)-2-metil-1,10-fenantrolin dan (1)-N-(4-benziloksi-3-metoksi-benzil)-1,10-fenantrolin klorida menunjukkan aktivitas antikanker *in vitro* yang kuat terhadap sel mieloma (NS-1) maupun Hela. Senyawa 4-kloro-3-(2-kloroetil)-2-metil-1,10-fenantrolin menunjukkan aktivitas antikanker yang kuat terhadap galur sel mieloma (NS1). Penelitian lebih lanjut akan dilakukan untuk mengevaluasi aktivitas antikanker *in vivo* pada model kanker hewan coba.

INTRODUCTION

Cancer is a disease in which there is uncontrolled multiplication and spread within the body of abnormal forms of the body's own cells. It is one of the major causes of death in the developed nations. In Europe and North America at least one in five of the population can expect to die due to cancer[1]. Worldwide cancer incidence is slated to double from 10 to 20 million per annum and the death rate is predicted to increase from 6-10 million by 2020[2]. As there are no epidemiological data in Indonesia, the exact incidence and prevalence of cancer are not known. However, data collected from hospitals in several regions shows that cancer incidence had increased by 2-8% per year during the last decade[3].

There are three main approaches to treat established cancer, surgical excision, irradiation and chemotherapy. Chemotherapy of cancer, as compared with that of bacterial disease, presents a difficult problem. In biochemical terms, microorganisms are both quantitatively and qualitatively different from human cells, however cancer cells and normal cells are so similar in many respects that it is more difficult to find general, exploitable, biochemical differences between them[1]. The development of drug resistance is also inevitable, so the discovery and exploitation of new cancer targets are very important[2].

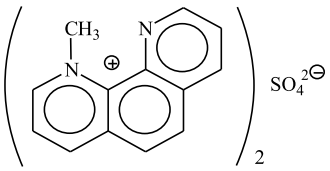
Tumour cells produce metalloproteinases and angiogenic factors that facilitate tumour growth, invasion of normal tissue and metastases. Targetting the mechanism, such as metalloproteinase inhibitor has the potential to be successful[1]. The 1,10-phenantrolines ring system is well known for its metalloproteinase inhibition activities by chelating divalent metal ions. As a chelating metal compound, 1,10-phenantroline has been used as antimicrobial agent against bacterial species such as *Prevotella ruminicola, Fibrobacter succinogenes, Lachnospira multipara* and *Megasphaera elsdenii*[4]. The complex of vanadyl-1,10-phenanthroline showed high antitumor activity for the human nasopharyngeal carcinomas cancer KB cell[5].

In our study concerning antiplasmodial activity of 1,10 phenanthroline derivatives, we found some compounds possessing a potential cytotoxic in normal cell line. Some compounds of *N*-alkyl and *N*-benzyl 1,10-phenanthroline derivatives showed antiplasmodial activity against FCR-3 and D10 *P. falciparum* with an IC_{50} value ranged from 0.10 to 3.60μM. However, some of these derivatives

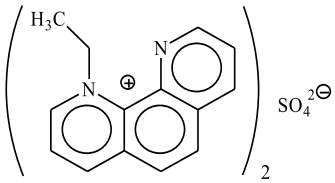
showed cytotoxic on normal Vero cell line with IC_{50} values + 168.81 µM and cytotoxic/antiplasmodial ratio + 24,19[6]. Based on these results, this study was conducted to know cytotoxic activity of 6 compound of *N*-alkyl and *N*-benzyl-1,10-phenanthroline derivatives that have been synthesized[7,8,9]. i.e. (1)-*N*-methyl-1,10-phenanthrolinium sulfate (1), (1)-*N*-ethyl-1,10-phenanthrolinium sulfate (2), 4-Bromo-3(2-bromoetyl)-2-metyl-1,10-phenantroline (3), 4-chloro-3(2-chloroetyl)-2-metyl-1,10-phenan-troline (4), (1)-*N*-benzyl-1,10-phenanthro-linium iodide (5), (1)-*N*-(4-benzyloxy-3-methoxy-benzyl)-1,10-phenanthrolinium chloride (6) on human cancer cell lines.

Materials and Methods

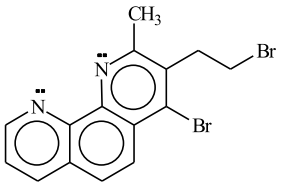
Six derivatives of 1,10-phenanthroline, 4 derivatives of *N*-alkyl and 2 derivatives of *N*-benzyl 1,10-phenanthroline were synthesized by Hadanu[7], Supargiyono *et al.*[8], and Mustofa *et al.*[9]. Doxorubicin HCl were purchased from Ferron Par Pharmaceuticals, Indonesia. The chemical structure of these compounds are shown in FIGURE 1.



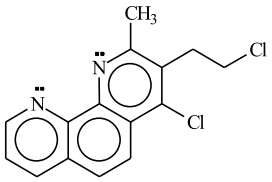
(1)-*N*-methyl-1,10-phenanthrolinium sulfate (1)



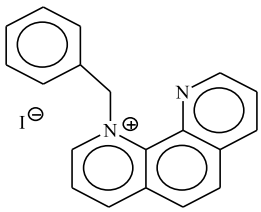
(1)-*N*-ethyl-1,10-phenanthrolinium sulfate (2)



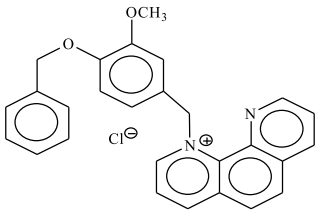
4-Bromo-3(2-bromoetyl)-2-metyl-1,10-phenantroline (3)



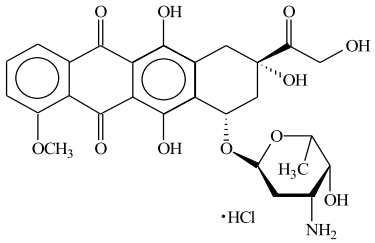
4-chloro-3(2-chloroetyl)-2-metyl-1,10-phenantroline (4)



(1)-*N*-benzyl-1,10-phenanthrolinium iodide (5)



(1)-*N*-(4-benzyloxy-3-methoxy-benzyl)-1,10-phenanthrolinium chloride (6)



Doxorubicin Hydrochloride (7)

FIGURE 1. Six derivatives of *N*-alkyl and *N*-benzyl-1,10-phenanthroline and doxorubicin HCl.

Cytoxicity of the compounds was assessed against two human cancer cell lines, myeloma cell line (NS-1, I Wayan Artama collection) and HeLa cell line (Indwiani Astuti collection). The cells were obtained from Integrated Research and Testing Laboratory, Gadjah Mada University, Indonesia. The human cancer cell lines were maintained *in vitro* in RPMI-1640 medium (Sigma-Aldrich Inc., USA), supplemented with 7.68 mM HEPES (Sigma Chemical Co., USA), 23.78 mM $NaHCO_3$ (Sigma-Aldrich Inc., USA), containing 10% fetal bovine serum (Gibco Invitrogen, USA), 2% of penicillin-streptomycine (Gibco Invitrogen, USA), and 0.5% of fungizon (Gibco Invitrogen, USA) in tissue culture flask. The cell was incubated in 5% of CO_2 incubator, 37°C. Trypan blue exclusion assay method[10] was used to evaluate the cytotoxicity of the compounds. The myeloma cells or HeLa cells were cultured in 96-well plates at 2 x 10^4 cells/well in 100 µL medium. One hundred µL of testing compounds or doxorubicin HCl solution as control added at various concentrations and were incubated for 24 h. The first concentration of the compound (1 mg/mL) was dissolved in dimethyl sulfoxide (DMSO) (Merck, Germany) and then was diluted with RPMI-1640 medium to obtain various concentrations of the compound. Cell growth was estimated by counting viable cell by hemocytometer and light microscope after 24 h incubation. The control cell free from any compounds was referred as 100% cell growth. Concentrations inhibiting 50% (IC_{50}) of cell growth were determined by probit analysis using *SPSS software*.

RESULTS AND DISCUSSION

The 1,10-phenantrolines ring system is well known for its metalloprotease inhibition activities by chelating divalent metal ions. As a chelating metal compound, 1,10-phenantroline has been used as antimicrobial agent against bacterial species such as *P. ruminicola, F. succinogenes, L. multipara M. elsdenii*[4]. The antimalarial activity of 1,10 phenanthroline was reported by Yapi *et al.*[11], in exhibiting better activity after blocking the potential chelating site by *N*-alkylation. Some compounds of *N*-alkyl and *N*-benzyl 1,10-phenanthroline derivatives showed antiplasmodial activity against FCR-3 and D10 *P. falciparum*[6] and in mouse malaria model[12].

The antitumor activity of 1,10-phenanthroline was reported by Sakurai *et al.*[5]. A derivative 1,10 phenanthroline, bis (4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium (IV) induced apoptosis in human cancer cells[13], exhibited antileukemic agent with matrix metalloproteinase inhibitor activity[14], significant antitumor activity and delayed tumor progression in CB.17 SCID mouse xenograft models of human glioblastoma and breast cancer[15].

The increased dependence of tumor cells on iron has led to the suggestion that depleting iron may represent a strategy to limit tumor growth[16]. Potent Fe chelator, 2-hydroxy-1-naphthylaldehyde isonicotinoyl hydrazone demonstrated selectivity against cancer cells compared to normal cells *in vitro*[17]. Metal chelators and metal ions have been shown to induce apoptosis through ROS generation[18] and as effective antiproliferative agents[19,20].

In this research, the chelating capacity of 1,10-phenantroline was blocked by *N*-10 alkylation and *N*-10 benzylation. Two human cancer cell lines, myeloma cell line (NS-1) and HeLa cell line were used to evaluate the *in vitro* cytotoxic activities of compound (1)-(6). The results are summarized in Table 1. The IC_{50} values of 1,10-phenanthroline derivatives ranged from 4.68 to 15.63 µM on myeloma cell and from 2.82 to 16.89 µM on HeLa cell. These results showed that only compound (5) that has IC_{50} value higher than doxorubicin. These results revealed that most of *N*-alkyl and *N*-benzyl 1,10-phenanthroline derivatives in this study have anticancer activity on myeloma and HeLa cell lines.

The compound (3) and (6) with IC_{50} values ranged from 4.68 to 4.72 µM on myeloma cell showed the same ($p>0.05$) cytotoxicity with doxorubicin with IC_{50} values ranged from 2.82 to while 3.08 µM on HeLa cell showed the higher ($p<0.05$) cytotoxicity than doxorubicin. It showed that compound (3) and (6) have same activity with doxorubicin in inhibiting myeloma cell growth, and have higher activity than doxorubicin in inhibiting HeLa cell growth.

The compound (4) with IC_{50} values 4.77 + 1.58 µM on myeloma cell showed the same cytotoxicity ($p<0.05$) with doxorubicin, however its IC_{50} values

TABLE 1. IC_{50} (μM) of *N*-alkyl and *N*-benzyl 1,10-phenanthrolines derivatives on myeloma (NS1) and HeLa cell lines

	Compound	IC_{50} (μM)	
		Myeloma (NS1) cell	HeLa cell
(1).	(1)-*N*-methyl-1,10-phenanthrolinium sulfate	8.87 ± 0.64	4.05 ± 0.86
(2).	(1)-*N*-ethyl-1,10-phenanthrolinium sulfate	15.63 ± 8.08	5.24 ± 0.48
(3).	4-Bromo-3(2-bromoetyl)-2-metyl-1,10-phenantroline	4.68 ± 1.25	2.82 ± 0.28
(4).	4-Chloro-3(2-chloroetyl)-2-metyl-1,10-phenantroline	4.77 ± 1.58	16.89 ± 0.18
(5).	(1)-*N*-benzyl-1,10-phenanthrolinium iodide	12.15 ± 1.31	8.57 ± 0.58
(6).	(1)-*N*-(4-benzyloxy-3-methoxy-benzyl)-1,10-phenanthrolinium chloride	4.72 ± 0.88	3.08 ± 0.96
(7).	Doxorubicin	2.39 ± 0.27	4.85 ± 0.31

on HeLa cell is higher than doxorubicin. It revealed that cytotoxic activity of this compound on myeloma cell is higher than that on HeLa cel

Among 6 compounds tested, 5 compounds showed more active against HeLa cell. The IC_{50} values of these compounds on Hela cell is lower ($p<0.05$) than on myeloma cell. Except, compound (4) that its IC_{50} values on myeloma cell is lower than on HeLa cell. This results showed that most of the compounds were more active on Hela cell than on myeloma cell.

In conclusion, the 4-Bromo-3(2-bromoetyl)-2-metyl-1,10-phenantroline(3) and (1)-*N*-(4-benzyloxy-3-methoxy-benzyl)-1,10-phenanthrolinium chloride (6) revealed potent *in vitro* anticancer activity on both myeloma (NS-1) and HeLa cells lines. The 4-chloro-3(2-chloroetyl)-2-metyl-1,10-phenantroline (4) revealed potent *in vitro* anticancer activity on myeloma (NS-1) cells line. Further study will be conducted to evaluate *in vivo* anticancer activity on animal cancer model.

ACKNOWLEDGEMENTS

This study was funded by Indonesian 10th Competitive Integrated Research (Riset Unggulan Terpadu X) from Minister of Research & Technology and 2nd Postgraduate Research Grant (Hibah Tim Pascasarjana II) from Minister of Education, Indonesian Government. Thanks also to Mrs. Rumbiwati for laboratory assistance.

REFERENCES

1. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology, 5th ed., Churchill Livingstone, Edinburg.
2. Workman P, New drug target for genomic cancer therapy: success, limitations, opportunities and future challenges. Current Cancer Drug Targets, 2001(1): 33-47
3. Tjindarbumi D, Mangunkusumo R, Cancer in Indonesia, resent and future. Jpn J Clin Oncol 2002;32 (Supplement 1): S17-S21.
4. Wallace RJ, Newbold CJ, McKain N. Inhibition by 1,10-phenantroline of the breakdown of peptides by rumen bacteria and protozoa. J Appl Bacteriol 1996; 80: 425-30.
5. Sakurai H, Tamura H, Okatani K. Mechanism for new antitumor vanadium complex:hydroxyl radical-dependent DNA cleavage by 1,10-phenanthroline-vanadyl complex in the presence of hydrogen peroxide. Biochem & Biophys Res Com 1995, 206(1):133-37.
6. Sholikhah EN, Supargiyono, Jumina, Wijayanti MA, Tahir I, Hadanu R, Mustofa. *In vitro* antiplasmodial activity and cytotoxicity of newly synthesized *N*-alkyl and *N*-benzyl-1,10-phenanthroline derivatives. Southeast Asian J Trop Med Public Health 2006; 37(6): 1072-77.
7. Hadanu R. Synthesis of (1)-*N*-alkyl-1,10-phenanthrolinium and 3-(2-Hydroxietyl)-2-methyl-1,10-phenanthroline-4-ol [thesis]. Yogyakarta: Postgraduate School, Gadjah Mada University, 2004.
8. Supargiyono, Jumina, Mustofa. New antimalarial of 1-phenyl-1,10-phenanthroline: synthesis, antiplasmodial activity, toxicity, pharmacokinetic, action mechanism and their formula. Minister of Education Indonesian Government: Research Report of 2nd Postgraduate Research Grant; 2004.
9. Mustofa, Jumina, Wijayanti MA, Tahir I. Sholikhah EN. Development of new compounds of 1,10-phenanthroline derivatives as antimalarial drug. Minister of Research and Technology Indonesian Government: Research Report of 10th Competitive Integrated Research, 2005.
10. Freshney RI. Culture of animal cells, a manual of basic technique. 4th ed. New York:Wiley-Liss, 2000.
11. Yapi AD, Mustofa, Valentin A, Chavignon O, Teulade JA, Mallie M, Chapat JP, Blache Y. New potential

antimalarial agents: synthesis and biological activities of original diaza analogs phenanthrene. Chem Pharm Bull 2000; 48(12): 1886-89.
12. Wijayanti MA, Sholikhah EN, Tahir I, Hadanu R, Jumina, Supargiyono, Mustofa. Antiplasmodial activity and acute toxicity of *N*-alkyl and *N*-benzyl-1,10-phenanthroline derivatives in mouse malaria model. J Health Sci 2006; 52(6): 794-99.
13. Narla, RK, Dong Y, D'Cruz OJ, Navara C, Uckun, FM. Bis (4,7-dimethyl-1,10-phenanthroline) sulfato-oxovanadium (IV) as a novel apoptosis-inducing anticancer agent. Clin Cancer Res 2000; 6: 1546-56.
14. Narla, RK, Dong Y, Klis D, Uckun, FM. Bis (4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium (IV) as a novel antileucemic agent with matrix metallo-proteinase inhibitor activity. Clin Cancer Res 2001; 7: 1094-101.
15. Narla, RK, Chen, CL, Dong Y, Uckun, FM. *In vivo* antitumor activity of bis (4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium (IV) {METVAN [$VO(SO_4)$ $(Me_2\text{-}Phen)_2$]}. Clin Cancer Res 2001; 7: 2124-33.
16. Huang X, Iron overload and its association with cancer risk in humans: evidenve for iron as carcinogenic metal. Mutat Res 2003; 533: 153-71.
17. Chaston TB, Lovejoy DB, Watts RN, Richardson DR. Examination of the antiproliverative activity of iron chelators: multiple cellular targets and the different mechanism of action of triapne compared with desferrioxamine and the potent pyridoxal isonicotinoyl hydrazone analoque 311. Clin Cancer Res 2003; 9: 402-14.
18. Duan H, Wang Y, Aviram M, Swaroop M, Loo JA, Bian J, Tian Y, Mueller T, Bisgaier CL, Sun Y. SAG, a novel zinc ring finger protein that protects cell from apoptosis induced by redox agents. Mol Cell Biol 1999; 19(4): 3145-55.
19. Darnell G, Richardson DR. The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents III: the effect of the ligands on molecular targets involved in proliferation. Blood 1999; 94(2):781-92.
20. Gao J, Richardson R. The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents, IV: the mechanism involved in inhibiting cell-cycle progression. Blood 2001; 98(3):842-50.