RESEARCH ARTICLE

Possible Anticancer Activity of Rosuvastatine, Doxazosin, Repaglinide and Oxcarbazepin

Fathia Zaky El Sharkawi[1]*, Hany Abdelaziz El Shemy[2], Hussein Moustafa Khaled[3]

Abstract

Background: Rosuvastatine, doxazosin, repaglinide and oxcarbazepin are therapeutic drugs available in the market for the treatment of different diseases. Potential to display antitumor activities has also been suggested. The aim of the current study was to evaluate their *in vitro* effects on some human transformed cell lines. Materials and Methods: Cytotoxicity of the four drugs was tested in MCF-7, HeLa and HepG2 cells by the neutral red assay method and also the effect of rosuvastatine and doxazosin against Ehrlich Ascities Carcinoma Cells (EACC) by trypan blue assay. Results: Rosuvastatine exerted the greatest cytotoxic effect against HepG2 cells with an IC_{50} value of 58.7±69.3; in contrast doxazosin showed least activity with IC_{50}=104.4 ±115.7. Repaglinide inhibited the growth of both HepG2 and HeLa cells with IC_{50} values of 87.6±117.5 and 89.3±119.5, respectively. Oxcarbazepine showed a potent cytotoxicity against both HeLa (IC_{50}=19.4±43.9) and MCF7 cancer cells ((IC_{50}=22±35.7).On the other hand the growth of EACC was completely inhibited by doxazosine (100% inhibition) while rosuvastatine had weak inhibitory activity (11.6%) . Conclusions: The four tested drugs may have cytotoxic effects against hepatic, breast and cervical carcinoma cells; also doxazosine may inhibit the growth of endometrial cancer cells. Further investigations in animals are needed to confirm these results.

Keywords: Rosuvastatine - doxazosin - repaglinide - oxcarbazepin - cell lines - in vitro anticancer chemosensitivity

Asian Pac J Cancer Prev, **15 (1)**, 199-203

Introduction

At present, cancer remains a significant health problem worldwide. Systemic therapy is an integral part of cancer management (Traxler, 2003). However the cost-effective benefit of most of the new drugs are under study, in view of the world wide efforts to rationalize the expenditure on health care. One of the ways to such rationalization on health expenditure is to try to make use of already available drugs that are currently not being used as cytotoxic agents to be explored as anticancer medications, or at least to potentiate the effects of the standard systemic therapy of cancer.

Statins, such as rosuvastatin and fluvastatin drugs are inhibitors of 3hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase , they are used in the treatment of hypercholesterolemia (Aguilar-Salinas et al., 1993). In addition, it has been reported that statins may display additional pharmacological properties such as antiviral,(Bader et al., 2008) and antitumor activities (Takahashi et al., 2006).However the antitumor molecular mechanisms by which the statin block cancer cell growth are poorly understood (Ghosh-Choudhury et al., 2010). So there are emerging interests to explore the anticancer potentials of HMG-CoA reductase inhibitors in the clinical setting, particularly in tumor sites sensitive to these agents in vitro (Chan et al., 2003).

Doxazosin is an oral drug that belongs to a class of drugs called alpha-1 adrenergic blockers. It is used for treating high blood pressure and symptoms of benign prostatic hyperplasia. Recently, the effect of a1-adrenoceptor antagonists on the apoptosis of both androgen dependent and androgen-independent prostate cancer cells has been investigated. Interestingly, several lines of evidence suggest that apoptotic effect is independent of the blockade of a1-adrenoceptors (Arencibia et al., 2005) .

Repaglininde is a new class of oral antidiabetic agents belonging to meglitinide family. It is absorbed rapidly, stimulates insulin release within a few minutes by inhibiting ATP-sensitive potassium channels of the beta-cell membrane via binding to a receptor distinct from that of sulphonylureas (Landgraf, 2000). It was reported that some antidiabetic drugs may have direct anti-tumor effects and have been shown to suppress various types of cancer cells in cell culture and in animal models (Feng et al., 2011).

*[1]Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, Helwan University , Helwan, [2]Department of Biochemistry, Faculty of Agriculture, [3]Department of Medical Oncology, National Cancer Institute (NCI), Cairo University, Giza, Egypt *For correspondence: felsharkawi@gmail.com*

Lastly oxcarbazepin is an antiepileptic drug used as a monotherapy or as an adjunctive therapy in the treatment of partial seizures. It was reported that it had an apoptotic and degenerative effects on rat uterine and ovarian cells (Cansu et al., 2010).

Based on this information , the aim of the present study was to evaluate the in vitro chemosensitivity of rosuvastatine, doxazosin, repaglinide and oxcarbazepine which are currently not being used as cytotoxic agents against three different solid tumor cell lines including HeLa (cervical cancer), HepG2 (hepatocellular carcinoma) and MCF-7cells (breast cancer) cell lines. In addition, the effects of rosuvastatine and doxazosin were also tested against Ehrlich Ascities Carcinoma Cells (EACC, endometrial cancer) cell line.

This would represent the first part of a series of experiments that aims to screen most of agents that could be of potential benefit for cancer patients.

Materials and Methods

Chemicals and drugs

RPMI-1640 media, fetal bovine serum, dimethyl sulfoxide (DMSO) solvent and other cell culture materials were purchased from Fisher Scientific Cell Culture (Houston, TX, USA). Neutral Red was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other reagents were of the highest analytical grade available.

Rosuvastatine, doxazosin, repaglinide and oxcarbazepin were obtained from Chemipharm, Pharaonia, Epico and Novartis Pharmaceutical companies respectively.

Cell culture

Human transformed cell lines, from liver (hepatocellular;HepG2), breast (MCF-7) and cervical (HeLa) cell lines were obtained from Vaccera (Giza, Egypt). Cells were maintained in RPMI-1640 supplemented with 100 µg/ml streptomycin, 100 µg /ml penicillin and 10% (w/v) heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO_2 atmosphere at 37°C.

Cytotoxicity assays

The cytotoxicity of rosuvastatine, doxazosin, repaglinide and oxcarbazepin was tested against MCF-7, HeLa and HepG2 cells by the neutral red assay as previously described (Repetto et al., 2008). Exponentially growing cells were collected using 0.25% Trypsin-EDTA and plated in 96-well plates at 1000-2000 cells/well. Cells were exposed to each test compound for 72 hrs in appropriate condition 5% (v/v) CO_2 atmosphere at 37°C. After several washings, cells were exposed to 0.0075% NR solution for 2 hours in humidified atmosphere of 5% (v/v) CO_2 at 37°C subsequently washed with PBS. Ethanol/ water/acetic acid (50:49:1) solution was used to dissolve the NR stained cells and color intensity was measured at 540 nm. in a micro-plate reader.

Determination of cell viability by trypan blue assay

Cytotoxicity of rosuvastatine and doxazosin was tested against Ehrlich Ascities Carcinoma cells (EACC) was employed as a representative of animal tumor cell lines. Animals were transplanted with EACC from an immortal culture obtained from National Cancer Institute, Cairo University, and maintained at mice transplanted line. International protocols governing the ethical treatment of animals were followed. EACC cell counts were adjusted to 106 cells/1 ml (counting both mature and immature cells).

The cytotoxicity of each compound against EACC cells was determined by the Trypan blue exclusion test (Bennett et al., 1976). The cell counts were adjusted to (10^5 cell/ 0.1 ml). Next ,0.1 ml of the cell suspension containing 10^5 cells/0.1 ml was added to each of four 1.8 ml screw-caps sterile Eppendorf tubes. Ten concentrations of the tested compounds were added, three replicas each. For each compound one tube served as negative control, where culture medium was added instead of the active compound. The tubes were incubated at 37°C in the presence of 5% (v/v) CO_2 for 24 hrs (dark condition, humidified air). After overnight incubation, cells were stained with Trypan blue (0.2%) dissolved in PBS, and the number of viable (unstained) versus dead (stained) cells was estimated. Two hundred cells were counted for EACC tube.

Statistical data

Data was represented by graphPad prism version 3.0. IC_{50} was calculated from the linear regression of the appropriate part of the perecent viability curve using the least square method. Significance difference between groups was assessed by 95% confidence limits (95%CL).

Results

Rosuvastatine, doxazosin, repaglinide and oxcarbazepin showed cytotoxicity effects against the three solid tumor cell lines. All these drugs inhibited cell growth with different potentiality.

Rosuvastatine had more cytotoxic effect against HepG2 cell line than the other two tested cell lines with IC_{50} value of 58.7±69.3 and 95%CL=29.4 to 88.1 at a concentration of 300ug/ml ; while its IC_{50} against MCF7 was 91.0±113.4 µg/ml and 95%CL=34.4 to 147.7. On the other hand it showed the least activity against HeLa cells with IC_{50}=106.0±77.6 and 95%CL=67.2 to 144.7 at the same tested concentration (Figure.1).

Doxazosin inhibited the proliferation of the three tested cell lines with different values; its IC_{50} against HeLa was 14.9± 48.5 and 95%CL=-9.3 to 39.1,while

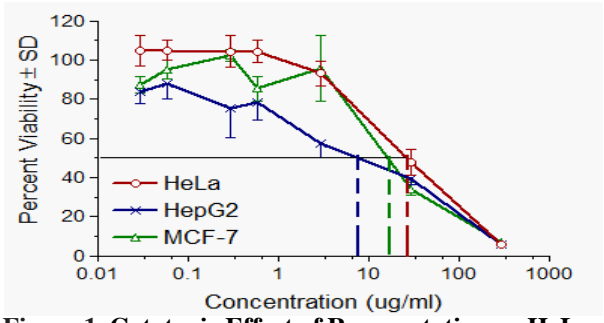

Figure 1. Cytotoxic Effect of Rosuvastatine on HeLa , HepG2 and MCF7 Cell Lines

Table 1. Cytotoxicisty Effects of Rosuvastatine, Doxazosin, Repaglinide and Oxcarbazepine Against HeLa, HepG-2, MCF-7 Cell Lines. 95%Confidence Limit was Used for Testing the Significance. A group mean to be Significantly Different than the other Can be seen by no Overlapping between the two 95%CLs; in other-words, the upper Limit of the 95%CL of the Smaller Mean is less than the Lower Limit of the 95% of the Larger mean ($p < 0.05$)

Substance		HeLa	HepG-2	MCF-7
Rosuvastatine	IC_{50}± SD, μg/ml	106.0±77.6#	58.7±69.3	91.0±113.4
	95% CL, μg/ml	67.2 to 144.7	29.4 to 88.1	34.4 to 147.7
Doxazosin	IC_{50}± SD, μg/ml	14.9± 48.5*	104.4±115.7	86.6±158.5
	95% CL, μg/ml	-9.3 to 39.1	46.6 to 162.2	7.4 to 165.8
Repaglinide	IC_{50}± SD, μg/ml	89.3±119.5	87.6±117.5	109.0±143.2
	95% CL, μg/ml	38.7 to 139.9	37.9 to 137.4	48.4 to 169.6
Oxcarbazepine	IC_{50}± SD, μg/ml	19.4±43.9	189.8±71.8**	22.5±35.7
	95% CL, μg/ml	-2.5 to 41.4	159.4 to 220.1	4.6 to 40.3

*Doxazosin is significantly more potent against HeLa than against HepG-2 cell line; **Oxcarbazepine is significantly less potent against HepG-2 than against other cell lines; #Rosuvastatine is significantly less potent against HeLa cell line than both Doxazosin and Oxcarbazepin

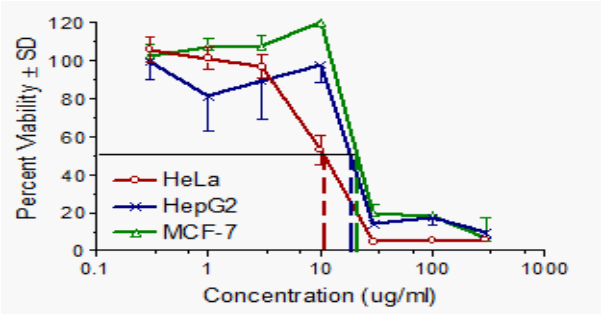

Figure 2. Cytotoxic Effect of Doxazosin on HeLa , HepG2 and MCF7 cell lines

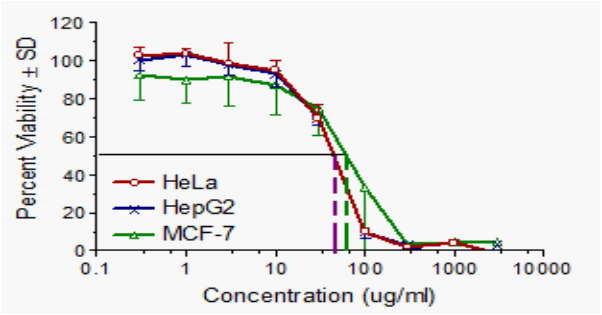

Figure 3. Cytotoxic Effect of Repaglinide on HeLa , HepG2 and MCF7 Cell Lines

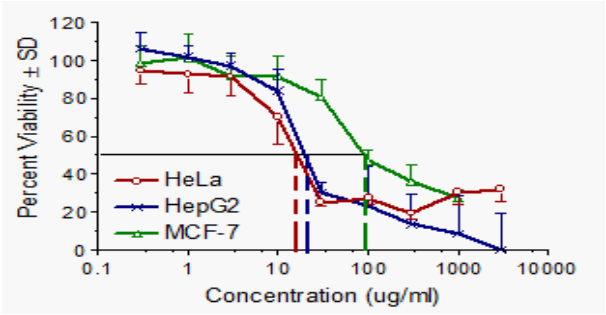

Figure 4. Effect of Oxcarbazepine on HeLa, HepG2 and MCF7 Cell Lines

on MCF7 it had IC_{50} of 86.6±158.5 and 95%CL = 7.4 to 165.8 at a concentration of 300 ug/ml. In contrast to rosuvastatine;HepG2 was the least susceptible cell line to doxazosin the IC_{50} was 104.4 ±115.7 with 95% CL= -9.3 to 162.2 at the tested concentration (Figure.2).

Repaglinide and oxcarbazepine were tested at a higher concentrations than that of rosuvastatine and doxazosine. Repaglinide showed an equi effectivety aginst both HeLa cells [IC_{50}=89.3±119.5 and 95%CL=38.7 to 139.9] and HepG2 cancer cells [IC_{50}=87.6±117.5 and 95%CL of 37.9 to 137.4]. While it was less potent against MCF7cells with IC_{50}=109 ±143.2 and 95%CL=48.4 to 169.6 at a tested concentration of 3000ug/ml (Figure. 3).

Oxcarbazepine showed more potent cytotoxicity against both HeLa and MCF7cells than HepG2 cells at a concentration of 3000ug/ml .The IC_{50} of the drug against HeLa cancer cells was 19.4±43.9 with 95%CL= -2.5 to 41.4; while that against MCF7cells was 22±35.7 with 95%CL= 4.6 to 40.3. Regarding HepG2 cells; the IC_{50} of the drug was 189.8±71.8 with 95%CLequall to 159.4 to 220.1 (Figurre 4).

Table 1 shows the different cytotoxicity effects of the four tested compounds against the three types of cell lines used .

Figures 1,2,3 and 4 represent the dose response curves of rosuvastatine, doxazosin, repaglinide and oxcarbazepine in solid tumor cell lines of HeLa, HepG2 and MCF7 cells.

Cell viability by trypan blue assay:

Doxazosine inhibited the growth of EACC cells more than rosuvastatine. It inhibited the growth of all the cells used (100% inhibition); while rosuvastatine inhibited 11.62% of cell growth at the concentrations used of 300ug/ml for the two drugs.

Discussion

The discovery of new applications for drugs with known clinical and toxicological profiles would cut down the time required for scaling-up to clinical application. The drugs used in the current study are clinically safe ; applied in treatment of different diseases and with cheaper prices rather than most chemotherapeutic or targeted agents now available for cancer patients. Here the cytotoxic profile of the chosen drugs was evaluated on three solid tumor cell lines, MCF-7, HeLa and HepG2. In addition the antiproliferative effects of two of these drugs were tested against EACC cell line .

A reduction in the availability of cholesterol may limit the cellular proliferation required for cancer growth and metastasis. Nielsen et al. (2012) tested the hypothesis

that statin use begun before a cancer diagnosis may be associated with reduced cancer-related mortality. So, they assessed mortality among 18,721 cancer patients who had used statins regularly before the cancer diagnosis and 277,204 who had never used statins. They found that statin use in patients with cancer is associated with reduced cancer-related mortality.

Statins increase peroxisome proliferator-activated receptor (PPAR) mRNA expression, but the mechanism of this increased PPAR production remains elusive. Majority of statins enhanced PPAR promoter activity in a dose-dependent manner in HepG2 cells transfected with the human PPAR promoter. This enhancement may be mediated by statin-induced HNF-4.

Rosuvastatin is one of the statins which increased PPAR mRNA expression in HepG2 cells after 24 hours treatment (Seo et al., 2008); While it had a poor cytotoxic effect against HeLa cells (Campos-Lara and Mendoza-Espinoza, 2011). Similar results were observed in the current study regarding the low cytotoxic activity of rosuvastatin against HeLa cells than HepG2 and MCF7 cells. Taken together, this may suggest a need for trials of statins in patients with cancer.

Doxazosin is the most widely investigated a1-adrenoceptor antagonist. Several signaling pathways have been identified to explain doxazosin-induced anoikis and cell apoptosis, namely through activation of transforming growth factor-b and InB pathways (Partin et al., 2003). inhibition of protein kinase B/Akt activation (Shaw et al., 2004); induction of death receptor-mediated apoptosis (Garrison and Kyprianou, 2006); increase in Bax expression (Chiang et al., 2005) and reduction in focal adhesion kinase (Walden et al., 2004). Doxazosin increased the apoptotic rate and total cell death rate of the HeLa cells in a dose-dependent manner and upregulated the expression of AP-2alpha and caspase-3 (Gan et al., 2008). In the present study the growth of HeLa cells was inhibited by a dose of 300 ug/ml of doxazosin and the same dose had a cytotoxic effect against MCF7cell lines with IC_{50}=86.6±158.5 and 95% CL=7.4 to 165.8 which could be correlated with the study of Hui et al. (2008) that reported the in vitro antiproliferative activity of doxazosin against breast cancer cells through the inhibition of EGFR and NF-kB signalling pathways . However, and unlike statins , there are no previous reports on possible effects of doxazosin use to reduce mortality in cancer patients.

Various antiepileptic drugs such as valproic acid, carbamazepine, oxcarbazepine, lamotrigine and levetiracetam are known to exert histone deacetylase inhibitory (HDACi) properties, which can modify aberrantly silenced gene expression by an epigenetic mechanism (Stettner et al., 2012). Histone deacetylases (HDACs) regulate the expression and activity of numerous proteins involved in both cancer initiation and cancer progression. By removal of acetyl groups from histones, HDACs create a non-permissive chromatin conformation that prevents the transcription of genes that encode proteins involved in tumorigenesis. In addition to histones, HDACs bind to and deacetylate a variety of other protein targets including transcription factors and other abundant cellular proteins implicated in control of cell growth, differentiation and apoptosis (Glozak and Seto, 2007). Theses findings may explain the inhibitory effects of oxcarbazepine on the proliferation of the three cancer cells used in the current study by different rates although its least activity against HepG2 cells .

Some antidiabetic drugs may have direct anti-tumor effects such as metformin and rosiglitazone which suppressed cancer cell growth and induced apoptosis of breast and pancreatic cancer cells (Feng et al., 2011) On the other hand Qian et al. (2008) reported that glibenclamide greatly decreased the cellular viability, induced apoptosis and inhibited Akt activation in wild-type mouse embryonic fibroblast (MEF) cells. Little is available about the in vitro chemosensitivity of repaglinide which observed here in the present study with closely similar IC_{50} values against both HeLa and HepG2 cells and with less activity against MCF7 cancer cells.

Although these in vitro concentrations of the four drugs tested in the present study were effective concentrations , it cannot be applied practically in vivo since the range of these concentrations are far higher than the peak plasma levels of these drugs when given to patients. However, using a different schedule of administration, e.g. metronomic low dose prolonged exposure schedule, or using these drugs as potentiating agents to other anticancer medications , may be possible for clinical application.

So in conclusion, rosuvastatine, doxazosin, repaglinide and oxcarbazepine have in vitro antiproliferative activity against HeLa, HepG2 and MCF-7 tumor cell lines with different values and activities. Further in vitro investigations on wider range of different cell lines e.g. NCI-60 panel and also using animal studies are needed to confirm the potential use of these drugs as anticancer agents.

Acknowledgements

The Authors acknowledge Dr. M El Mazar, Prof. of Pharmacology, Dean of Faculty of Pharmacy ,The British university in Egypt for his role in graphically presenting the data , statistical analysis and its interpretation.

References

Aguilar-Salinas CA, Barrett H, Schonfeld G (1993). Metabolic modes ofaction of the statins in the hyperlipoproteinemias. *Atherosclerosis*, **141**, 203-7.

Arencibia JM, Del Rio M, Bonnin A, et al (2005). Doxazosin induces apoptosis in LNCaP prostate cancer cell line through DNA binding and DNA-dependent protein kinase down-regulation. *Int J Oncol*, **27**, 1617-23.

Bader T, Fazili J, Madhoum M, et al (2008). Fluvastatin inhibits hepatitis C replication in humans. *Am J Gastroenterol*, **103**, 1383-9.

Bennett JM, Catovsky D, Danniel MT, et al (1976). Proposal for the classification of the acute leukemia. *Br J Heam*, **33**, 451-8.

Campos-Lara M and Mendoza-Espinoza JA (2011). Cytotoxic evaluation of fluvastatin and rosuvastatin and effect of fluvastatin in the hela cell cycle. *African J Pharm Pharmacol*, **5**, 189-93.

Cansu A, Erdogan D, Serdaroglu A, et al (2010). Histologic and morphologic effects of valproic acid and oxcarbazepine on

rat uterine and ovarian cells. *Epilepsia*, **51**, 98-107.
Chan KWK, Oza MA, Siu LL (2003). The statins as anticancer agents. *Clin Cancer Res*, **9**, 10-19.
Chiang CF, Son EL,Wu GJ (2005). Oral treatment of the TRAMP mice with doxazosin suppresses prostate tumor growth and metastasis. *Prostate*, **64**, 408-18.
Feng YH, Velazquez-Torres G, Gully C, et al (2011). The impact of type 2 diabetes and antidiabetic drugs on cancer cell growth. *J Cell Mol Med*, **15**, 825-36.
Garrison JB , Kyprianou N (2006). Doxazosin induces apoptosis of benign and malignant prostate cells via a death receptor-mediated pathway. *Cancer Res*, **66**, 464-72.
Gan L, Zhu DX, Yang LP, et al (2008). Involvement of transcription factor activator protein-2alpha in doxazosin-induced HeLa cell apoptosis. *Acta Pharmacol Sin*, **29**, 465-72.
Ghosh-Choudhury N, Mandal CC, Ghosh-Choudhury G (2010). Simvastatin induces derepression of PTEN expression via NFkappaB to inhibit breast cancer cell growth. *Cell Signal*, **22**, 749-58.
Glozak MA, Seto E (2007). Histone deacetylases and cancer . *Oncogene*, **26**, 5420-32.
Hui H, Fernando MA, Heaney AP (2008). The alpha1-adrenergic receptor antagonist doxazosin inhibits EGFR and NF-kappaB signaling to induce breast cancer cell apoptosis. *Eur J Cancer*, **44**, 160-6.
Landgraf R (2000). Meglitinide analogues in the treatment of type 2 diabetes mellitus. *Drugs Aging*, **17**, 411-25.
Nielsen SF, Nordestgaard B G, Bojesen SE (2012). Statin use and reduced cancer-related mortality. *N Engl J Med*, **367**, 1792-802.
Partin JV, Anglin IE, Kyprianou N (2003). Quinazoline-based a1 adrenoceptor antagonists induce prostate cancer cell apoptosis viaTGF-b signaling and I kappa B a induction. *Br J Cancer*, **88**, 1615-21.
Qian X, Li J, Ding Jm, et al (2008) .Glibenclamide exerts an antitumor activity through reactive oxygen species-c-jun NH2-terminal kinase pathway in human gastric cancer cell line MGC-803. *Biochem Pharmacol*, **76**, 1705-15.
Repetto G, Peso A , Zurita J (2008). Neutral red uptake assay for the estimation of cell viability /cytotoxicity. *Nature Protocols*, **3**, 1125-31.
Seo M, Inoue I, Ikeda M, et al (2008). Statins activate human PPAR promoter and increase PPAR mRNA expression and activation in HepG2 cells. *PPAR-Res*, **2008**, 11.
Shaw YJ, Yang YT, Garrison JB, et al (2004). Pharmacological exploitation of the a1-adrenoreceptor antagonist doxazosin to develop a novel class of antitumor agents that block intracellular protein kinase B/Akt activation. *J Med Chem*, **47**, 4453-62.
Stettner M, Krämer G, Strauss A, et al (2012). Long-term antiepileptic treatment with histone deacetylase inhibitors may reduce the risk of prostate cancer. *Eur J Cancer Prev*, **21**, 55-64.
Takahashi HK, Weitz-Schmidt G, Iwagaki H, et al (2006) . Hypothesis: The antitumor activities of statins may be mediated by IL18. *J Leukoc Biol*, **80**, 215-6.
Traxler P (2003). Tyrosine kinases as targets in cancer therapy - successes and failures. *Expert Opin Ther Targets*, **7**, 215-34.
Walden PD, Globina Y, Nieder A (2004). Induction of anoikis by doxazosin in prostate cancer cells is associated with activation of caspase 3 and a reduction of focal adhesion kinase. *Urol Res*, **32**, 261-5.