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MOLECULAR EFFECTS OF AMINE DERIVATIVES OF PHENOTHIAZINE ON CANCER CELLS C-32 AND SNB-19 *IN VITRO**

MAŁGORZATA LATOCHA[1]**, ANDRZEJ ZIĘBA[2], RENATA POLANIAK[3], DARIUSZ KUŚMIERZ[1], ADA NOWOSAD[1], MAGDALENA JURZAK[4], EWA ROMUK[5], MIROSŁAWA KOKOCIŃSKA[1] and ELEKTRA SLIUPKAS-DYRDA[1]

[1] Department of Cell Biology, Medical University of Silesia in Katowice, School of Pharmacy with the Division of Laboratory Medicine, Jedności 8, 41-200 Sosnowiec, Poland
[2] Department of Organic Chemistry, Medical University of Silesia in Katowice, School of Pharmacy with the Division of Laboratory Medicine, Jagiellońska 4, 41-200 Sosnowiec, Poland
[3] Department of Human Nutrition, Medical University of Silesia in Katowice, School of Public Health, Jordana 19, Zabrze 41-808, Poland
[4] Department of Cosmetology, Medical University of Silesia in Katowice, School of Pharmacy with the Division of Laboratory Medicine, Kasztanowa 8, 41-200 Sosnowiec, Poland
[5] Department of General Biochemistry, Medical University of Silesia in Katowice, School of Medicine with the Division of Dentistry, Jordana 19, 41-808 Zabrze, Poland

Abstract: Cancer therapy is challenging for scientists because of low effectiveness of so far existing therapies (especially in case of great invasiveness and advanced tumor stage). Such need for new drug development and search for more efficient new findings in therapeutical applications is therefore still valid. There are also conducted studies on modifying so far existing drugs and their new methods of usage in oncology practice. One of them is phenothiazine and its derivatives which are used in psychiatric treatment for years. They also exhibit antiprion, antiviral, antibacterial and antiprotozoal properties. Cytotoxic activity, influence on proliferation, ability to induce apoptosis suggest also a possibility of phenothiazine derivatives usage in cancer cells termination. The aim of our the study was to evaluate the influence of two amine derivatives of phenothiazine on cancer cells *in vitro*. Amelanotic melanoma C-32 cell line (ATCC) and glioma SNB-19 cells (DSMZ) were used in this study and two derivatives were analyzed. In view of examined substances tumor potential toxicity cells proliferation and viability exposed to phenothiazine derivatives were established. Cell cycle regulatory genes expression (*TP53* and *CDKN1A*), S-phase marker - H3 gene and intracellular apoptosis pathway genes (*BAX, BCL-2*) were analyzed using RT-QPCR method. The influence of examined derivatives on total cell oxidative status (TOS), total antioxidative status (TAS), malondialdehyde concentration (MDA) and superoxide dismutase activity (SOD) were analyzed. As a result, examined phenothiazine derivatives cytotoxic action on C-32 and SNB-19 and also cells proliferation inhibition were determined. Cell cycle regulatory genes (*TP53, CDKN1A*) expression and protein products of genes involved in mitochondial apoptosis pathway (*BAX, BCL-2*) expression are changed by the presence of phenothiazine derivatives during culturing. There were also noted small changes in redox potential in cells exposed to two mentioned phenothiazine derivatives.

Keywords: phenothiazine derivatives, C-32, SNB-19

Abbreviations: *BAX* - pro-apoptotic BAX protein gene, *BCL-2* - anti-apoptotic BCL-2 protein gene, *CDKN1A* - cyclin-dependent kinase inhibitor 1 (or CDK-interacting protein 1) gene, *H3* - H3 histone gene (S-phase marker, proliferation indicator), MDA - malondialdehyde, Real-Time RT-QPCR - Real Time Reverse Transcription Quantitative Polymerase Chain Reaction, SOD - superoxide dismutase activity,TOS - total oxidative status, *TP53* - gene of the cell cycle regulatory P53 protein

Cancer is still very significant therapeutical problem. Rising number of oncology patients leads scientists and doctors to search for new therapeutical options including great interest in looking for new drugs and substances with antiproliferative and cytotoxic actions directed to tumor cells. Difficulties in this field derives from tumor cells themselves. These are resistant to apoptosis, often indicate multi-drug resistance and lowered oxygen demand. Additionally, impeded drug delivery system in

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**Corresponding author: email: mlatocha@sum.edu.pl; phone: +48 32 364 12 11

tumors caused by incorrect vascular construction, interstitial pressure and pH changes is observed. Because of mentioned factors and problems, new anticancer drugs are sought. These substances are often modified existing anticancer drugs with changed chemical structure and mode of action.

Great interest in cancer treatment is laid in phenothiazines and their derivatives. This group includes aliphatic, piperazine, piperidine trycyclic compounds, where two benzene rings are bound by sulfur and nitrogen atoms. Phenothiazines exhibit an antipsychotic-neuroleptic actions, antihistamine, antitussive and antimetic actions, also antiprion, antiviral, antibacterial and antiprotozoal activity (1-3). It was also observed that patients treated with phenothiazines less likely suffer from cancer (4). Some of phenothiazine derivatives indicate preventive antitumor actions (their biological anticancerogenous activity) by viruses destruction (5-9). Moreover, phenothiazines can indicate antiproliferative activities by calmodulin antagonists activity, which binds calcium ions and takes part in cells proliferation induction (10). Phenothiazines can also act as both pro- and antioxidants (11, 12). It was indicated that some of these derivatives inhibit transporting functions of P-glycoprotein, causing better ability to penetrate directly by cytostatics into tumor cells and affect them (2, 13). They may also have pro-apoptotic properties of deciding on the intracellular ceramide content, which stimulates accession to apoptosis (14). It was proved that phenothiazine derivatives actions and effectiveness depend on kind and location of substituent in the particular compound chemical structure (2, 8, 15, 16). For example, -CF_3 substituent in phenothiazine derivatives in C2 phenothiazine ring position is characterized as highly proapoptotic while substances with -Cl atom in the same C2 position act much less proapoptotic (fluphenazine is classified as highly proapoptotic drug and chlorpromazine is classified as slightly proapoptotic compound). Nowadays, research are focused on new derivatives with higher proapoptotic activity and with precisely directed antitumor actions (17).

The aim of the present study was to examine two amine derivatives of phenothiazine impact on tumor cells in cell culture conditions.

EXPERIMENTAL

Cell culture

In the study, amelanotic melanoma C-32 (ATCC, USA) and glioma SNB-19 (DSMZ, Germany) cells were analyzed. Cells were cultured by using DMEM medium (Lonza, Switzerland) with 10% fetal bovine serum (FBS) (Biological Industries, Israel) and penicillin (1000 units/mL) with streptomycin (10 mg/mL) mixture (Biological Industries, Israel).

Phenothiazine derivatives

Derivatives of phenothiazine used were: A) 9-(*N*-piperidyl)-5-methyl-12(*H*)-quino[3,4-*b*][1,4]benzothiazinium chloride and B) 9-amino-5-methyl-12(*H*)-quino[3,4-*b*][1,4]benzothiazinium chloride (Fig. 1). They were synthesized in the Department of Organic Chemistry (Medical University of Silesia in Katowice, School of Pharmacy with the Division of Laboratory Medicine).

Cytotoxicity

Cells viability was analyzed by C-32 and SNB-19 culturing in chosen phenothiazine derivatives concentrations (0.1, 0.5, 1, 5, 10, 50 and 100 µg/mL). The number of cells in cultures was evaluated after 72 h of incubation with the test derivatives in media without the addition of FBS. WST-1 test (Roche Diagnostics GmbH, Germany) was used to examine viability cells number and LDH (Roche Diagnostics GmbH, Germany) test was used to examine dead cells number. Absorbance analyses were made by using UVM340 microplate reader (Biogenet, Polska).

Proliferative activity

The influence of phenothiazine derivatives on cells proliferative activity was evaluated after cells incubation by 72 h with examined compounds in media with FBS (Biological Industries, Israel) con-

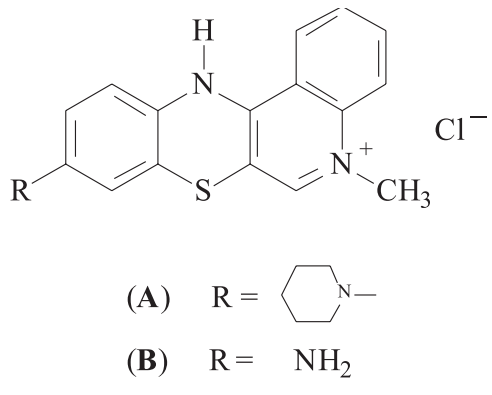

Figure 1. Derivatives of phenothiazine tested. A - 9-(*N*-piperidyl)-5-methyl-12(*H*)-quino[3,4-*b*][1,4]benzothiazinium chloride; B - 9-amino-5-methyl-12(*H*)-quino[3,4-*b*][1,4]benzothiazinium chloride

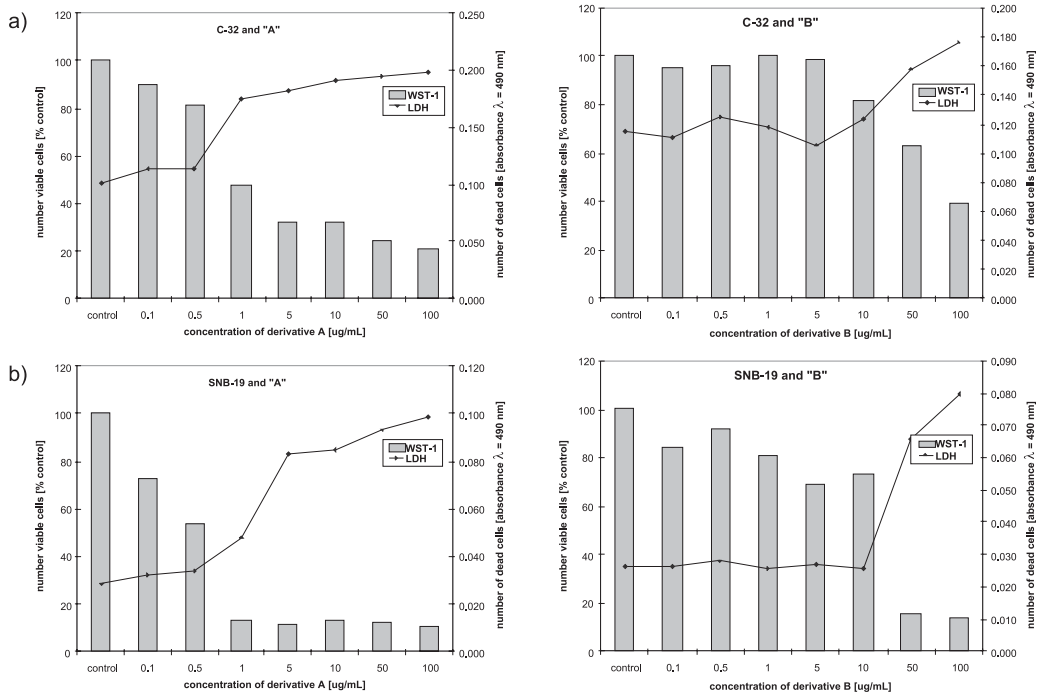

Figure 2. Cytotoxicity of A and B phenothiazines - number of cells in cultures of C-32 (a) and SNB-19 (b) after 72 h exposure to phenothiazine derivatives A and B. The number of viable cells (% of control) (WST-1 test) and the number of dead cells in the cultures (absorbance $\lambda = 490$ nm) (LDH assay). Statistical significance is indicated with star ($p \leq 0.05$).

tent. To establish cell number, WST-1 test (Roche Diagnostics GmbH, Germany) was used. Absorbance analysis were made by using UVM340 microplate reader (Biogenet, Poland).

Genes transcriptional activity: *H3, BCL-2, BAX, TP53, CDKN1A*

Genes trancriptional activity was evaluated by real time RT-QPCR method with OPTICON TM DNA Engine (MJ Research, USA) and QuantTect® SYBR® Green RT-PCR Kit (Qiagen GmbH, Germany). Genes analyzed were coding: histone H3 (*H3*), mitochondrial apoptosis pathway proteins (BCL-2 (*BCL-2*), BAX (*BAX*)) and cell cycle regulator protein P53 (*TP53*) and P21 (*CDKN1A*). Cells were exposed to examined phenothiazine derivatives in 0.5 µg/mL concentration for 24 h. RNA extraction was made using Quick-RNA™ Kit MiniPrep (ZYMO RESEARCH, USA). Quality and quantity of extracts was established. Total RNA integrity was analyzed in 1.2% agarose (PRONA S.A., Spain) electrophoresis with added ethidium bromide compound by using electrophoresis and blotting system - Mini-PROTEAN Tetra Cell and Mini Trans-Blot Module (BioRad, USA). Extracts total RNA quantity and purity were established using spectrophotometric analysis with HP845 spectrophotometer (Hewlett Packard, USA).

Antioxidative enzymes activity

Cells *in vitro* were exposed to examined phenothiazine derivatives in 0.5 µg/mL concentration for 24 h. As a next step, cells were harvested and lysed. After centrifugation, cell supernatant was stored on ice to analyze its total oxidative status (TOS) (18), superoxide dismutase activity (SOD) [EC 1.15.1.1] and malondialdehyde (MDA) concentration, which is lipids peroxidation indicator. SOD activity was measured by Oyanagui method (19), using its different susceptibility to cyanide inhibition, expressed as nitro units per gram of protein content. MDA concentration was measured by Ohkawa at al. method (20), using thiobarbituric acid reaction and results were shown as µmol of MDA per gram of protein content (21).

Statistical analysis

Statistical analysis was made using Statistica PL 9.0. Cell number in cell cultures and examined genes mRNA copy number after PCR reaction were

shown as an average of three replicates of three consecutive experiments with standard deviation (SD) content. Analysis used *t*-Student test for variables not dependent samples, Fisher test was used to count variance p factor, distribution normality was analyzed by Shapiro-Wilk test. Statistically significant changes were established on $p < 0.05$ relevance level.

RESULTS

Cell number analysis result in cell cultures exposed by 72 h to examined phenothiazine derivatives are shown in Figures 2 and 3. The examined phenothiazine derivatives cytotoxicity effect is visualised in Figure 2a (living and dead C-32 cell number in cell cultures exposed to A and B phenothiazine derivatives influence for 72 h) and Figure 2b (living and dead SNB-19 cell number in cell cultures exposed to A and B phenothiazine derivatives influence for 72 h). In both C-32 and SNB-19 cells, phenothiazine derivative A indicates more significant cytotoxic effect what WST-1 and LDH tests results confirm. SNB-19 have shown metabolically active cells level significant decrease starting with A derivative in 0.1 µg/mL concentration (WST-1). Also starting with 1 µg/mL derivative A concentration affecting SNB-19 cells, dead cell number increase (LDH) was observed. As described above, also melanoma C-32 cells indicate decreased viability and dead cell number increase while incubated with phenothiazine derivative A starting with 1 µg/mL concentration. In parallel, examined derivative B indicated cytotoxic effect starting with 50 µg/mL concentration (SNB-19) and 10 µg/mL concentration (C-32). Examined compounds affect cells proliferative ability by its significant decrease – derivative A already at a concentration of 0.1 µg/mL (C32 and SNB-19), derivative B at a concentration of 50 ug/mL (C32 and SNB-19) (Fig. 3). WST-1 analysis results seem to confirm those obtained by PCR analysis for phenothiazine derivatives in concentration 0.5 µg/mL, where *H3* histone (proliferation indicator) gene trancriptional activity decreases. Noticeable *H3* gene expression increase in SNB-19 examined with derivative B can derive from applied concentration in analyzed samples (Table 1). Copy number mRNA of analyzed *TP53* gene did not shown any significant changes between C-32 samples. In SNB-19 cells, derivative B presence caused significant *TP53* expression increase (Table 1). Phenothiazine B derivative also affected lowering P21 (*CDKN1A*) gene mRNA copy number in both C-32 and SNB-19 cells (Table 1). Despite visible cytotoxic effects in cultures exposed to A and B derivatives, any characteristic apoptotic genes *BAX* and *BCL-2* activity changes have not been observed (Table 1). Changes observed in increased ratio *BAX*/*BCL-2* mRNA copy number in examined samples, suggest cells protective antiapoptotic mechanisms activation (Table 1).

Preliminary cells TOS analysis results with phenothiazine A and B derivatives with 0.5 µg/mL supplemented media and MDA analysis results in both (melanoma nad glioma cells) indicate ongoing oxidative processes in examined samples (Table 2). Particularly evident are A derivative actions in C-32 cultures. SOD increased activity can be a confirmation of cell oxidative status changes for tested A and B compounds. The mentioned changes in SOD activity as one of major antioxidative enzymatic components, can also be a proof for protective

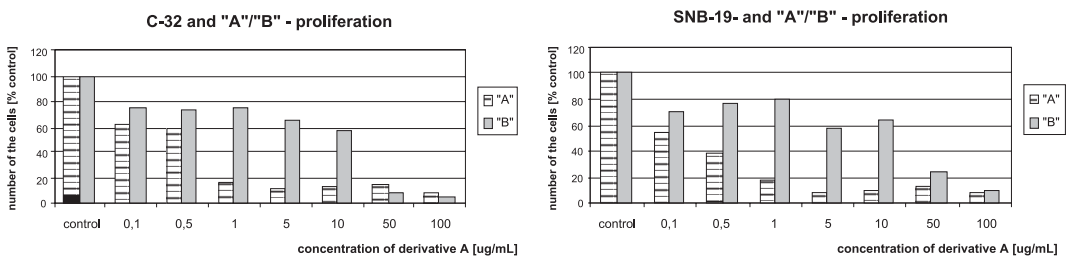

Figure 3. The proliferative activity of cells (C32 and SNB-19) - number of cells after 72 h exposure to the A and B derivatives of phenothiazine. Statistical significance is indicated with star ($p \leq 0.05$).

Table 1. A and B phenothiazine derivatives influence on *H3, TP53, CDKN1A* and *BCL-2, BAX* genes expression.

Gene	C-32			SNB-19		
	Number of mRNA copies/µg total RNA					
	Control	"A"	"B"	control	"A"	"B"
H3	183 ± 29	72 ± 15	123 ± 27	2436 ± 248	605 ± 53	56210 ± 4388
TP53	2892 ± 512	3130 ± 1302	2392 ± 283	1348 ± 293	3510 ± 751	144951 ± 21364
CDKN1A	3968 ± 732	5108 ± 964	660 ± 78	3294 ± 290	6500 ± 781	1605 ± 30
BCL-2	38675 ± 1652	68243 ± 23180	66416 ± 4258	2918 ± 396	4109 ± 799	17275 ± 3493
BAX	7350 ± 1652	2086 ± 848	3053 ± 571	26195 ± 4278	34356 ± 1355	53409 ± 7278
BCL-2/BAX	**5.26**	**32.71**	**21.75**	**0.111**	**0.120**	**0.323**

Table 2. Effect of phenothiazine A and B on the redox balance - TOS, MDA, SOD.

Gene	C-32			SNB-19		
	µmol/mg total protein					
	Control	"A"	"B"	control	"A"	"B"
TOS	0.82 ± 0.32	2.35 ± 1.11	1.96 ± 0.92	2.87 ± 0.85	3.53 ± 1.33	3.01 ± 1.53
MDA	24.33 ± 5.52	28.12 ± 8.52	25.34 ± 4.22	7.79 ± 2.31	14.62 ± 4.21	12.50 ± 7.32
	NU/mg total protein					
SOD	0.419 ± 0.122	1.280 ± 0.345	0.935 ± 0.423	0.383 ± 0.142	0.585 ± 0.131	0.461 ± 0.234

actions of SNB-19 and C-32 cells under examined conditions.

DISCUSSION and CONCLUSION

The aim of the study was to evaluate the influence of two amine derivatives of phenothiazine: A) 9-(*N*-piperidyl)-5-methyl-12(*H*)-quino[3,4-*b*][1,4]benzothiazinium chloride and B) 9-amino-5-methyl-12(*H*)-quino[3,4-*b*][1,4]benzothiazinium chloride on C-32 (ATCC) and SNB-19 (DSMZ) tumor cells *in vitro*. The analyzed molecules differ from each other by additional presence of quinobenzothiazinium chloride in amine group and piperidyl group at the 9- position of the quinobenzothiazinium ring.

Characteristic tumor cells feature is immortality and great proliferative activity which derives from impaired cell cycle regulation and control. As so far, existing knowledge says that phenothiazine derivatives can act as both cytotoxic and cytostatic compounds (2, 6, 7, 22-24). The examined cytotoxicity analysis of A and B derivatives have shown that in C-32 and SNB-19 cells more effectively acts A derivative (Fig. 2). WST-1 analysis was made to evaluate mitochondrial enzymes activity by specifying living cells number in cultures with media without FBS content. Such conditions prevented cell proliferation and where necessary for LDH analysis, where LDH enzyme is released by dead cells. First vital functions limitations effects were shown using 0.1 µg/mL derivatives concentration and 1 µg/mL concentration indicated statistically important dead cells number (Fig. 2). Fot both, A and B phenothiazine derivatives antiproliferative effect was also demonstrated. However, A substance indicated actions at lower concentration than B derivative (Fig. 3) in C-32 and SNB-19 cells. One of the anticancer drugs mechanisms of action is interaction with DNA what impedes proper cell metabolism and proliferation. Drugs affecting genes expression result in fully blocked replication. The mentioned mechanism was indicated by anthracycline antibiotics (doxorubicin) because of flat aromatic and heteroaromatic structural fragments. Also, so far existing knowledge suggests such antiproliferative effect in case of phenothiazines examinations (25, 26). In the present study, evaluated *in vitro* two chemical compounds A and B are phenothiazines analogues. Quinobezothiazinum salts X-ray analysis showed flat tetracycline structural compound (27), which attenuates intercalation process. Amine substituent present in the mentioned molecule may cause drug-DNA complex stabilization, what is possible due to

additional interactions of amino group *via* hydrogen bonds with purine and pyrimidine bases in the DNA molecule.

Antiproliferative activity of tested compounds for the C-32 cells has been confirmed in the analysis of expression of a gene encoding the histone H3 (Table 1). It is a generally accepted marker of proliferation in molecular studies. For SNB-19 cells, derivative B has increased the number of transcripts H3 (Table 1). Note, however, that the concentration of 0.5 ug/mL was used in these experiments (cytotoxic and antiproliferative effects appeared only at high concentrations - Figs. 2 and 3). The choice of concentration originated from the idea of comparing another experimental results not discussed it this study and also, A derivative concentration was limited by strong cytotoxic effect resulting in low living cells number at higher concentrations.

In the study, also BCL-2 (*BCL-2*), BAX (*BAX*), P53 (*TP53*) and P21 (*CDKN1A*) genes transcriptional activity was analyzed (Table 1). Real time RT-QPCR was made with QuantTect® SYBR® Green RT-PCR Kit (Quiagen) usage. Cells, as by histone *H3* analysis, were exposed to A and B derivatives in 0.5 µg/mL concentration.

TP53 gene product is P53 protein which acts as genome guardian and is also involved in many cell processes regulation. One of this protein major tasks is stopping cell cycle while DNA damages occur and when they are not repaired, P53 potein induce apoptosis. In cells, apoptosis can be initiated by intrinsic mitochondrial pathway with BCL-2 involved (pro- and antiapoptotic proteins family) or by extrinsic mitochondrial one were DR4 and DR5 receptors play an important role. P53 intracellular amount is strictly regulated by MDM2 protein (28). New target in anticancer therapies is to restore P53 activity in tumor cells what could lead to cancer cells precise degradation. This protein product regulates also protein P21 gene (*CDKN1A*) expression. This protein selectively binds to cyclin-dependent kinase complexes with cyclins and regulates cell cycle what means that inadequate P21 protein number or its mutations in cells can induce oncogenic transformation (29, 30). *TP53* and *CDKN1A* genes expression analysis performed in this study indicated significant increase of *TP53* expression only in SNB-19 glioma cells and phenothiazine derivative B examination (Table 1). It has been also shown that B derivative decreases mRNA copy number of P21 protein (*CDKN1A*). Obtained results and data give us new sight at actions of examined phenothiazine derivatives observed in cell cultures. However, it is important to draw attention that study applies only to examined genes mRNA transcripts. Facing changes in the expression of more genes, obtained results may not be precise. Some facilitation in the results interpretation are *BAX* and *BCL-2* genes expression analysis where *BAX*/*BCL-2* ratio is most important. Gene expression changes (among others P53 dependant) in proapoptotic *BAX* and antiapoptotic *BCL-2* ones do not indicate mitochondrial pathway cell number decrease dependence in cell cultures exposed to A and B derivatives. BAX protein role in cells lies in mitochondrial membrane permeability increase by pores formation therein, while BCL-2 protein is responsible for cytochrome C release into the cytosol (31, 32). As a result of described study, increased *BCL-2*/*BAX* ratio indicates protective mechanisms induction by examined cells before apoptosis pathway activation (Table 1).

The examined redox balance also has great importance for any cell proper functioning in living cells. Any disorders in this homeostasis can lead to oncogenic transformation but can be also used as a tool for its destruction and elimination from the organism (33-35). As obtained results have shown, C-32 exposition to A phenothiazine derivative (0.5 µg/mL) cause significant TOS changes and promotes oxidative activity (increased MDA concentration) in examined cells. However, SNB-19 indicate oxidative promotion by examined substances on lower level than in C-32 cells (Table 2). Also, SOD increased activity can confirm oxidative cell status in phenothiazine derivatives presence because of its great involvement in antioxidative protective actions as one of major enzymatic systems.

In conclusion, A and B phenotiazine derivatives influence tumor cells viability and chosen molecular markers in C-32 and SNB-19 cells. Analyzed molecules differ from each other by additional presence of quinobeznothiazinium chloride in amine group and piperidyl group at the 9-position of the quinobenzothiazinium ring. Both analogues cause multiple metabolic changes in C-32 and SNB-19 cells, what results in viability and proliferation limitations. Although A derivative indicated stronger effect of action, both A and B analogues seem not to affect BCL-2/BAX signalling pathway irregularities. Study results have shown that amine heterocyclic compound in quinobenzothiazinium chloride molecule increases antiproliferative activity in both examined cell lines. This result is very interesting and encourages studies focusing on the new qiuonobenzothiazinium salts synthesis as new potential anticancer drugs.

REFERENCES

1. Baral P.K., Swayampakula M., Rout M.K., Kav N.N, Spyracopoulos L. et al.: Structure 22, 291 (2014).
2. Jaszczyszyn A., Gąsiorowski K., Świątek P., Malinka W., Cieślik-Boczula K. et al.: Pharmacol. Rep. 64, 16 (2012).
3. Kostecka M.: Acta Pol. Pharm. Drug Res. 68, 137 (2011).
4. Fond G., Macgregor A., Attal J., Larue A., Brittner M. et al.: Med. Hypotheses 79, 38 (2012).
5. Abuhaie C.M., Bîcu E., Rigo B., Gautret P., Belei D. et al.: Bioorg. Med. Chem. Lett. 23, 147 (2013).
6. Diaconu I., Cerullo V., Escutenaire S., Kanerva A., Bauerschmitz G.J. et al.: J. Gene Med. 12, 435 (2010).
7. Chamoun-Emanuelli A.M., Pecheur E.I., Simeon R.L., Huang D., Cremer P.S., Chen Z.: Antimicrob. Agents Chemother. 57, 2571 (2013).
8. Pluta K., Morak-Młodawska B., Jeleń M.: Eur. J. Med. Chem. 46, 3179 (2011).
9. Fond G., Macgregor A., Attal J., Larue A., Brittner M. et al.: Med. Hypotheses 79, 38 (2012).
10. Khan S.Z., Longland C.L., Michelangeli F.: Biochem. Pharmacol. 60, 1797 (2000).
11. Nagaraja N., Kumar V., Vuppugundur V.: Der Pharmacia Lettre 4, 786 (2012).
12. Borges M.B., Dos Santos C.G., Yokomizo C.H., Sood R., Vitovic P. et al.: Free Radic. Res. 44, 1054 (2010).
13. Spengler G., Takács D., Horváth A., Riedl Z., Hajós G. et al.: Anticancer Res 34, 1737 (2014).
14. Jaszczyszyn A., Gąsiorowski K., Świątek P., Malinka W.: Onkol. Pol. 14(2), 43 (2011).
15. Baciu-Atudosie L., Ghinet A., Farce A., Dubois J., Belei D., Bîcu E.: Bioorg. Med. Chem. Lett. 22, 6896 (2012).
16. Abuhaie C.M., Bîcu E., Rigo B., Gautret P., Belei D. et al.: Bioorg. Med. Chem. Lett. 23, 147 (2013).
17. Berchtold M.W., Villalobo A.: Biochim. Biophys. Acta 1843, 398 (2014).
18. Erel O.: Clin. Biochem. 38, 1103 (2005).
19. Oyanagui Y.: Anal. Biochem. 142, 290 (1984).
20. Ohkawa H, Ohishi N, Yagi K.: Anal. Biochem. 95, 351 (1979).
21. Lin Y., Jamieson D.: Pharmacol. Toxicol. 70, 271 (1992).
22. Zięba A., Latocha M., Sochanik A.: Med. Chem. Res. 22, 4158 (2013).
23. Prinz H., Chamasmani B., Vogel K., Böhm K.J., Aicher B. et al.: J. Med. Chem. 54, 4247 (2011).
24. Zong D., Zielinska-Chomej K., Juntti T., Mörk B., Lewensohn R., Hååg P., Viktorsson K. Cell Death Dis. 5, e1111 (2014).
25. Hossain M., Giri P., Kumar G.S.: DNA Cell Biol. 27, 81 (2008).
26. Hossain M., Kumar G.S.: Mol. Biosyst. 5, 1311 (2009).
27. Zięba A., Sochanik A., Szurko A., Rams M., Mrozek A., Cmoch P.: Eur. J. Med. Chem. 45, 4733 (2010).
28. Allen M.A., Andrysik Z., Dengler V.L., Mellert H.S., Guarnieri A. et al.: Elife 3, e02200 (2014).
29. Mirzayans R., Andrais B., Scott A., Murray D.: J. Biomed. Biotechnol. 2012, 170325, (2012).
30. Sznarkowska A., Olszewski R., Zawacka-Pankau J.: Postepy Hig. Med. Dosw. 64, 396 (2010).
31. Karch J., Kwong J.Q., Burr A.R., Sargent M.A., Elrod J.W. et al.: Elife 2, e00772 (2013).
32. Zhu A.K., Zhou H., Xia J.Z., Jin H.C., Wang K. et al.: Braz J Med Biol Res 46, 670 (2013).
33. Sosa V., Moliné T., Somoza R., Paciucci R., Kondoh H,. Leonart M.E.: Ageing Res Rev 12(1), 376 (2013).
34. Casas A., Di Venosa G., Hasan T., Batlle A.: Curr. Med. Chem. 18, 2486 (2011).
35. Manda G., Nechifor M.,T., Neagu T-M.: Curr. Chem. Biol. 3, 342 (2009).

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