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The activity of a new 2-amino-1,3,4-thiadiazole derivative 4ClABT in cancer and normal cells

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Abstract: The 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole set are well known compounds with interesting *in vitro* and *in vivo* anti-cancer profiles. The aim of this study was an *in vitro* evaluation of the anti-cancer activity of a new synthesized aminothiadiazole derivative 2-(3-chlorophenyloamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole 4ClABT. The effect on tumor cell proliferation, motility and morphology, DNA synthesis as well as the influence on normal cells was assessed. The antiproliferative activity of 4ClABT in tumor cells derived from peripheral cancers including breast carcinoma (T47D), colon carcinoma (HT-29), thyroid carcinoma (FTC-238), teratoma (P19), and T-cell leukemia (Jurkat E6.1), as well as cancers of the nervous system including rhabdomyosarcoma/medulloblastoma (TE671), brain astrocytoma (MOGGCCM) and glioma (C6) was studied by means of MTT assay. DNA synthesis level was determined in BrdU ELISA test. Wound assay model was applied for tumor cell motility assessment. Morphological changes induced by 4ClABT in cancer and normal cells were analyzed in HE staining specimens. Moreover, the influence of 4ClABT on normal cells including skin fibroblasts (HSF), hepatocytes (Fao), astroglia and neurons was studied by means of LDH assay. The tested compound inhibited the proliferation of tumor cells in dose-dependent fashion. The anti-cancer effect was attributed to decreased DNA synthesis, prominent changes in tumor cell morphology as well as reduced cell motility. In antiproliferative concentrations, 4ClABT was not toxic to normal cells. Our study showed prominent anti-cancer effects of the tested aminothiadiazole derivative in the absence of toxicity in normal cells. The obtained results confirmed the promising anti-cancer profile of previously tested 2-(monohalogenphenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole derivatives (ClABT — chlorophenyl derivative, FABT and 3FABT — fluorophenyl derivatives and 4BrABT — bromophenyl derivative). The molecular mechanisms and the *in vivo* activity of aminothiadiazole derivatives will be the subject of further studies. (*Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 3, pp. 436–444*)

Key words: 2-amino-1,3,4-thiadiazole, cancer and normal cells, anti-cancer activity, cell proliferation, cell motility

Introduction

Aminothiadiazoles are a series of compounds with a wide spectrum of biological activity, including antimicrobial, antiviral, antifungal, antituberculosis, anti-inflammatory, anticonvulsant and anti-cancer properties [1–8]. Their ability to inhibit cancer development has been documented in numerous *in vitro* [7–11] and *in vivo* [12–18] studies. Their mechanism of action differs, depending on the type of modification of the thiadiazole ring [1, 19–23]. During the second half of the 20th century many different derivatives were synthesized based on this structure. The objects of our interest are 2-amino-1,3,4-aminothiadiazole derivatives.

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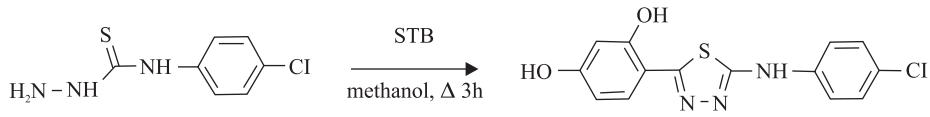

Figure 1. Synthesis scheme of 2-(4-chlorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (4ClABT)

In our previous publications, we have described the synthesis and anti-cancer activity of the members of 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-aminothiadiazole set against various cancer cell lines [24–29]. We have demonstrated the ability of the derivatives to inhibit cancer cell proliferation and motility, as well as decrease DNA synthesis. It is important to note that the tested compounds in anti-cancer concentrations had no influence on the viability of normal cells [28, 29]. Moreover, a prominent neuroprotective activity of 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (FABT) has been observed in the neuronal cultures exposed to neurodegenerative agents [28].

On the basis of QSAR analysis, we aspired to prepare a compound with a preferred anti-cancer profile, high binding efficiency and minimum toxicity. Nevertheless, the side-effects associated with cancer chemotherapy are still a serious problem limiting treatment possibilities. Some 2-amino-1,3,4-thiadiazole derivatives were introduced into phase II clinical trials in patients with ovarian, cervical, colon, leiomyosarcoma, and other types of cancer. Unfortunately, their application in treatment was limited by side-effects such as stomatitis, hyperuricemia, leucopenia, thrombocytopenia, nausea and vomiting [14–18]. However, 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-aminothiadiazole synthesized in our research showed relatively low toxicity for normal cells in *in vitro* culture conditions.

The objective of the present study was the evaluation of the activity of 4ClABT in cancer and normal cells.

Material and methods

Chemical characteristics of 4ClABT. ***Preparation of compound.*** The compound was obtained from sulfinyl bis(2,4-dihydroxythiobenzoyl) (STB) [30] and 4-(3-chlorophenyl)-3-thiosemicarbazide (Lancaster, Germany) or 4-(4-chlorophenyl)-3-thiosemicarbazide (Lancaster, Germany) via the endocyclization process [24] (Figure 1).

Analytical studies. The melting point (m.p.) was determined on a BUCHI B-540 (Switzerland) melting point apparatus. Elemental analysis was performed in order to determine C, H and N contents (Perkin-Elmer 2400; USA). The analyses were within ± 0.4% of the theoretical values. The vibration spectra were recorded with a Perkin-Elmer FT-IR 1725X (USA) spectrophotometer in KBr. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX 500 instrument. Chemical shifts (δ, ppm) were given with tetramethylsilane (TMS). The spectra MS (EI, 70 eV) were recorded using the apparatus AMD-604 (AMD Intectra GmbH, Germany).

2-(4-chlorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (4ClABT)

Yield: 69%; mp: 249–251°C. ^{1}H NMR (500 MHz, DMSO-d_6, δ): 6.40–6.42 (dd, J = 8.5 Hz and 2.3 Hz, 1H, C_5-H), 6.44 (d, J = 2.3 Hz, 1H, C_3-H), 7.37–7.39 (d, J = 8.9 Hz, 2H, $C_{2',6'}$-H), 7.67–7.70 (d, J = 8.9 Hz, 2H, $C_{3',5'}$-H), 7.79–7.80 (d, J = 8.6 Hz, 1H, C_6-H), 9.89 (s, 1H, NH), 10.37 (s, 1H, C_4-OH), 10.85 (s, 1H, C_2-OH); ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 102.43, 108.12, 108.45, 118.72, 124.84, 128.55, 128.81, 139.77, 155.01 (C_{thia}-5), 155.68, 160.32, 163.21 (C_{thia}-2); IR (KBr, cm^{-1}): 3,314, 3,250 (OH, NH), 1,625 (C = N), 1,601 (C = C), 1,221 (C-OH), 1,105 (C-Cl), 1,011 (N = C-S-C = N), 673 (C-S-C); EI-MS (m/z, %): 319 (M^+, 100), 184 (30), 167 (5), 152 (6), 153 (8), 149 (13), 135 (7), 94 (9), 75 (4), 66 (4). Anal. calcd. for $C_{14}H_{10}ClN_3O_2S$ (319.77): C, 52.59; H, 3.15; N, 13.14. Found: C, 52.38; H, 3.14; N, 13.20.

Biological studies. ***Cell lines.*** Human leukemic T-cell lymphoblast Jurkat E6.1 (Cat. No. 88042803), human breast carcinoma T47D (Cat. No. 85102201), human colon adenocarcinoma HT-29 (Cat. No. 91072201), human follicular thyroid carcinoma FTC-238 (Cat. No. 94060902), human rhabdomyosarcoma/medulloblastoma TE671 (Cat. No. 89071904), human brain astrocytoma MOGGCCM (Cat. No. 86022702) and rat hepatoma cells Fao (Cat. No. 89042701) were obtained from the ECACC (European Collection of Cell Cultures, A Health Protection Agency Culture Collection, Salisbury, UK). Mouse teratoma P19 (Cat. No. CRL-1825) was obtained from the ATCC (American Type Culture Collection, Manassas, VA, USA). Rat glioma (C6) was obtained from the Department of Neonatology, Charite-Virchow Clinics, Humboldt University, Berlin, Germany. Human skin fibroblasts (HSF) were obtained via the outgrowth technique from skin explants of young persons. Mouse neurons were differentiated from P19 cells by way of retinoic acid induction. Rat astroglia was isolated from the brains of suckling rat pups.

The following culture media purchased from Sigma (Sigma Chemicals, St. Louis, MO, USA) were applied: DMEM (C6, HSF), 1:1 mixture of DMEM and Nutrient mixture F-12 Ham (TE671, T47D, HT-29), 3:1 mixture of DMEM and

Nutrient mixture F-12 Ham (MOGGCCM) and RPMI (Jurkat E6.1), as well as AlphaMEM (P19) and F12 Nutrient Mixture Kaighn's (Fao) from Gibco, Life Technologies, Milan, Italy. All media were supplemented with 10% FBS (Sigma), penicillin (100 U/mL) (Sigma) and streptomycin (100 μg/mL) (Sigma). The cultures were kept at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

Neuronal cell culture. P19 cells were cultured in AlphaMEM with the addition of 10% FBS. For induction of the neuronal differentiation, cells were resuspended in AlphaMEM medium supplemented with 5% FBS and 0.5 μM retinoic acid at a density of 1×10^7 cells $\times$ mL^{-1}. Cells were incubated for 96 h on Petri dishes (the medium was changed for a fresh one after 48 h). The obtained neurospheres were aspirated and centrifuged at 450 rpm for 5 mins, rinsed with the medium without FBS and centrifuged again. Neurospheres were dispended using 0.01% trypsin-EDTA solution (10 mins, 37°C) and treated with 0.01% DNAase I in the presence of 10% FBS to obtain a single cell suspension. The cells were then sieved through a 40 μM cell strainer (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA). Finally, the neurons suspension was centrifuged at 800 rpm for 10 mins and resuspended in culture medium containing Neurobasal medium (Gibco), 2% B-27 supplement (Gibco), 2 mM L-glutamine (Sigma) and antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin). The cells were plated at a density of $4 \times 10^5 \times$ mL^{-1} on 96-well microplates (Nunc) coated with poly-L-lysine and kept at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The culture medium was changed every three days. The experiment was carried out ten days after culture establishment.

Astroglia culture. The primary astrocytes culture was established from the cortices of three-day-old Wistar rats. The brain tissue was pooled into ice cold glucose (33 mM) Hanks' Balanced Salt Solution (HBSS, Sigma), cut into small pieces and incubated for 30 mins at 37°C with 0.25% trypsin-EDTA solution. Next, single cells suspension was obtained, as described for neurons culture. The cells were inoculated into 75 cm^2 cell culture flasks and kept at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The cell culture medium contained a 1:1 mixture of DMEM and Nutrient mixture F-12 Ham supplemented with 10% FBS and antibiotics (penicillin and streptomycin). The medium was changed every two days until the culture reached confluency. Next, the flasks were shaken overnight in an orbital shaker at 210 rpm in order to remove less adherent cells (neurons, microglia and oligodendroglia). Following this shaking procedure, the culture became enriched with flat cells displaying typical astrocyte morphology. Immunostaining with a primary antibody for glial fibrillary acidic protein (GFAP, polyclonal, DAKO, Denmark) revealed that astrocytes accounted for ~95% of the cells in the culture.

Viability assay. Tumor cells were plated on 96-well microplates at a density of 0.1×10^4 (P19), 0.5×10^4 (C6), 1×10^4 (T47D, TE671, FTC238, MOGGCCM) and 3×10^4 (HT-29) $\times$ mL^{-1}. Different cell densities were used depending on the cells' size and growth rate. Densities were selected to obtain confluency in control culture after 96 h. The next day, the culture medium was removed and the cells exposed to serial dilutions of 4ClABT (1, 2.5, 5, 10, 25, 50 and 100 μM) in a fresh medium. In the Jurkat cells culture, 4ClABT was given intermediary to the cell suspension at a density of $2 \times 10^4 \times$ mL^{-1}. Cell proliferation was assessed after 96 h using the MTT method in which the yellow tetrazolium salt (MTT) is metabolized by viable cells to purple formazan crystals. The tumor cells were incubated for 3–4 h with MTT solution (5 mg/mL). Formazan crystals were solubilized overnight in SDS buffer (10% SDS in 0.01N HCl) and the product quantified spectrophotometrically by measuring absorbance at the 570 nm wavelength using a Elx800 microplate reader (BIO-TEK, Highland Park, Winooski, VT, USA).

Proliferation assay. Cells were plated on 96-well microplates at a density of 1×10^4 (C6) and 2×10^4 (T47D, FTC238) and 5×10^4 (HT-29) $\times$ mL^{-1}. Cell densities were selected depending on the cells' size and proliferation rate to obtain confluency in control culture after 48 h. The next day, the culture medium was removed and the cells exposed to serial dilutions of 4ClABT (1, 2.5, 5, 10, 25, 50 and 100 μM) in fresh medium. Cell proliferation was quantified after 48 h by measurement of BrdU incorporation during DNA synthesis (Cell Proliferation ELISA BrdU, Roche Diagnostics GmbH, Penzberg, Germany). The test was performed according to the manufacturer's instruction. The absorbance was measured at the 450 nm wavelength using a Elx800 microplate reader.

Cell migration assessment. Tumor cell migration was assessed by the wound assay model. Glioma C6 cells were plated at 1×10^6 cells on 3-cm culture dishes (Nunc). The next day, the cells were scratched by a pipette tip (P300), the medium and dislodged cells were aspirated, and plates rinsed twice with PBS. Next, the fresh culture medium was applied and the number of cells migrated into the wound area after 24 h was estimated in the control and the cultures treated with 4ClABT (5 and 10 μM). The plates were stained using the May-Grünwald-Giemsa method. The observation was performed by a Olympus BX 51 System Microscope (Olympus Optical Co., Ltd, Tokyo, Japan) and the micrographs prepared by analysis software (Imaging Software Olympus cell*Family). Cells migrated to the wound area were counted on micrographs and the results expressed as a mean cell number migrated to the selected 40 wound areas taken from four micrographs.

Table 1. Comparison of antiproliferative effect of 4ClABT in tumor cell lines (MTT assay). IC_{50} was calculated using computerized linear regression analysis of quantal log dose-probit functions according to the method of Litchfield and Wilcoxon [31]

Cell culture	Histotype	IC_{50} [μM] 4ClABT
FTC238	Thyroid carcinoma	6.4
HT-29	Colon carcinoma	6.5
JurkatE6.1	T-cell leukemia	6.7
P19	Teratoma	8.5
T47D	Breast carcinoma	10.7
C6	Glioma	12.7
TE671	Rhabdomyosarcoma-medulloblastoma	15.3
MOGGCCM	Astrocytoma	19.4

Cytotoxicity assay. Cells were plated on 96-well microplates at a density of 1×10^5 (HSF, astrocytes) and 2.5×10^5 (Fao) and 4×10^5 (neurons) $\times$ mL^{-1}. Cell densities were selected depending on the cells' size to obtain culture confluency the next day. The following day, the culture medium was removed and the cells exposed to 4ClABT (1, 2.5, 5, 10, 25, 50 and 100 μM) diluted in a fresh culture medium with a reduced amount of FBS (2%). Neurons were cultured in medium with addition of 2% B12 supplement. Cytotoxicity was detected using an In Vitro Toxicology Assay Kit, Lactic Dehydrogenase based (Sigma). The assay was based on the reduction of NAD by the action of lactic dehydrogenase (LDH) released from damaged cells. The resulting NADH was utilized in stoichiometric conversion of a terazolium dye. The resulting colored compound was measured spectrophotometrically. The test was carried out according to the kit procedure. The color product was quantified spectrophotometrically at 490 nm wavelength using an Elx800 microplate reader.

Cells morphology. Breast and thyroid carcinoma cells as well as normal fibroblasts at a density of 5×10^4 (T47D, FTC238) and 5×10^5 (HSF) $\times$ mL^{-1} plated on Lab-Tek Chamber Slide (Nunc) were exposed to 4ClABT (5, 10 and 25 μM) in medium with a reduced amount of FBS (2%). After 48 h, incubation cells were stained using the Hematoxylin-Eosin (HE) method. The observation was performed by an Olympus BX 51 System microscope (Olympus Optical Co., Ltd, Tokyo, Japan) and micrographs were prepared by analysis software (Imaging Software Olympus cell*Family).

Results

2-(4-chlorophenylamino)-5-(2,4-dihydroxyphenyl)--1,3,4-thiadiazole (4ClABT) (Figure 1) was obtained according to the procedure described previously [24, 30].

The anti-cancer effect of the tested compound was assessed in a range of tumor cell lines, including tumors derived from peripheral cancers (breast carcinoma, colon carcinoma, thyroid carcinoma, teratoma, T-cell leukemia), as well as cancers of the nervous system (rhabdomyosarcoma/medulloblastoma, brain astrocytoma and glioma). Tumor cells were exposed to either a culture medium (control) or 1, 2.5, 5, 10, 25, 50 and 100 μM of 4ClABT for 96 h and MTT assay was performed. On the basis of obtained results, IC50 values for each cell line (Table 1) were calculated (linear regression analysis of quantal log dose-probit functions according to the method of Litchfield and Wilcoxon [31]). The tested compound produced a 50% viability decrease of tested cancer cells in the concentration range of 6.4–19.4 μM. The most sensitive for 4ClABT appeared to be thyroid carcinoma FTC238 cells and the most resistant were brain astrocytoma MOGGCCM cells. A higher activity of 4ClABT was observed against tumor cells derived from peripheral cancers than cancer cells originating from the nervous system.

The effect of 4ClABT on tumor cells was attributed to decreased DNA synthesis, as determined by measurements of incorporation of BrdU (Figure 2). T47D, HT-29, FTC238 and C6 cells were treated with 4ClABT and medium only (control) for 48 h, and then BrdU assay was performed. ELISA analysis showed a concentration-dependent decrease of BrdU incorporation during DNA synthesis in cells treated with 4ClABT, in comparison to control cells.

In order to evaluate the influence of the tested aminothiadiazole derivative on cancer cell motility, the wound assay test was performed (Figure 3). Glioma C6 cell monolayers were wounded and exposed to 4ClABT for 24 h. The cells were then stained and analyzed in a light microscope. The results (Figure 3A) revealed a 27% (5 μM 4ClABT) and a 42% (10 μM 4ClABT) decrease in the number of cells migrated to the wound area compared to the control.

The effect of 4ClABT on normal human cells was tested by means of LDH assay. Human skin fibroblasts (HSF), rat hepatocytes (Fao), rat primary astroglia and mouse neurons were exposed to the 4ClABT (5–100 μM). Next, the level of lactic dehydrogenase (LDH) released from damaged cells was measured after 24 h (HSF, Fao, astrocytes) or 48 h (neurons) treatment. From our experimental experience, neuronal cell cultures require longer (48 h) incubation with tested substance or neurodegenerative agent (e.g. serum deprivation, excitotoxicity) to observe any remarkable neurotoxic or neurotrophic effect. For this reason, in neuronal culture a longer incubation time was used.

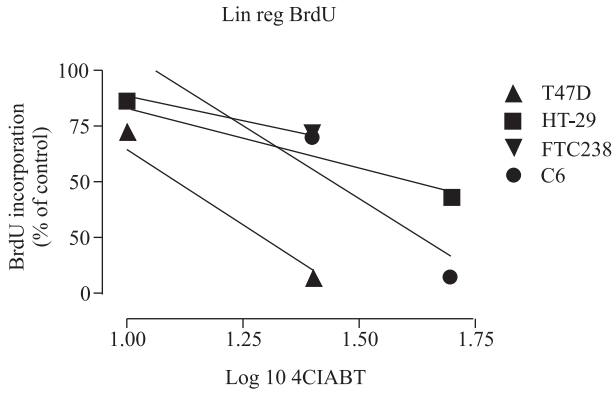

Figure 2. The antiproliferative effect of 4ClABT was attributed to decreased cell division. Human breast carcinoma (T47D), colon carcinoma (HT-29), follicular thyroid carcinoma (FTC238) cells and rat glioma (C6) cells were grown in culture medium only (control) and in the presence of tested compounds (1–100 μM) for 48 h. BrdU incorporation was used as a marker of cell division. The data represents mean normalized optical densities ± SEM of 3–6 trials and was analyzed by means of linear regression

The obtained results (Figure 4) indicated a lack of toxic influence of 4ClABT in anti-cancer concentrations on the normal cell cultures (skin fibroblasts, hepatocytes, astrocytes and neurons). Only at very high 4ClABT concentrations (50–100 μM) was a significant increase in the LDH level observed. Moreover, in the cultures of nervous cells (astrocytes and neurons), a significant decrease of the LDH level was observed compared to the control (Figures 4C, D).

The influence of 4ClABT on cell morphology was assessed in cancer and normal cells. Breast carcinoma T47D, thyroid carcinoma FTC238 and primary culture skin fibroblasts HSF were treated with the tested compound for 48 h and then stained using the

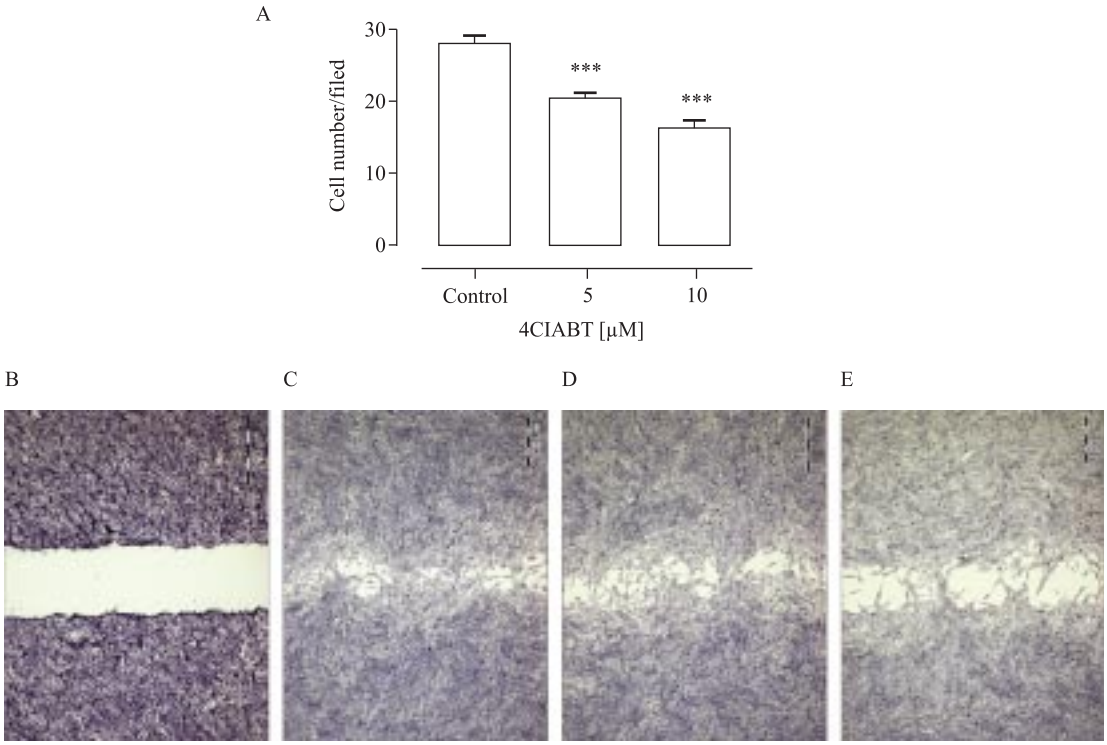

Figure 3. Effect of 4ClABT on the migration of glioma C6 cells. Wounded monolayers of glioma cells were incubated alone (control) and in the presence of 4ClABT (5, 10 μM) for 24 h. Columns show cell number migrated on the wound area in the control and culture treatment with 4ClABT (**A**), ***at least $p < 0.001$, vs. control, One-way ANOVA test, *post-hoc* Tukey. Micrograph shows wound assay of C6 cells, wound (**B**), cell migration after 24 h in control culture (**C**), exposure to 5 μM 4ClABT (**D**) and 10 μM 4ClABT (**E**). Magnification × 40

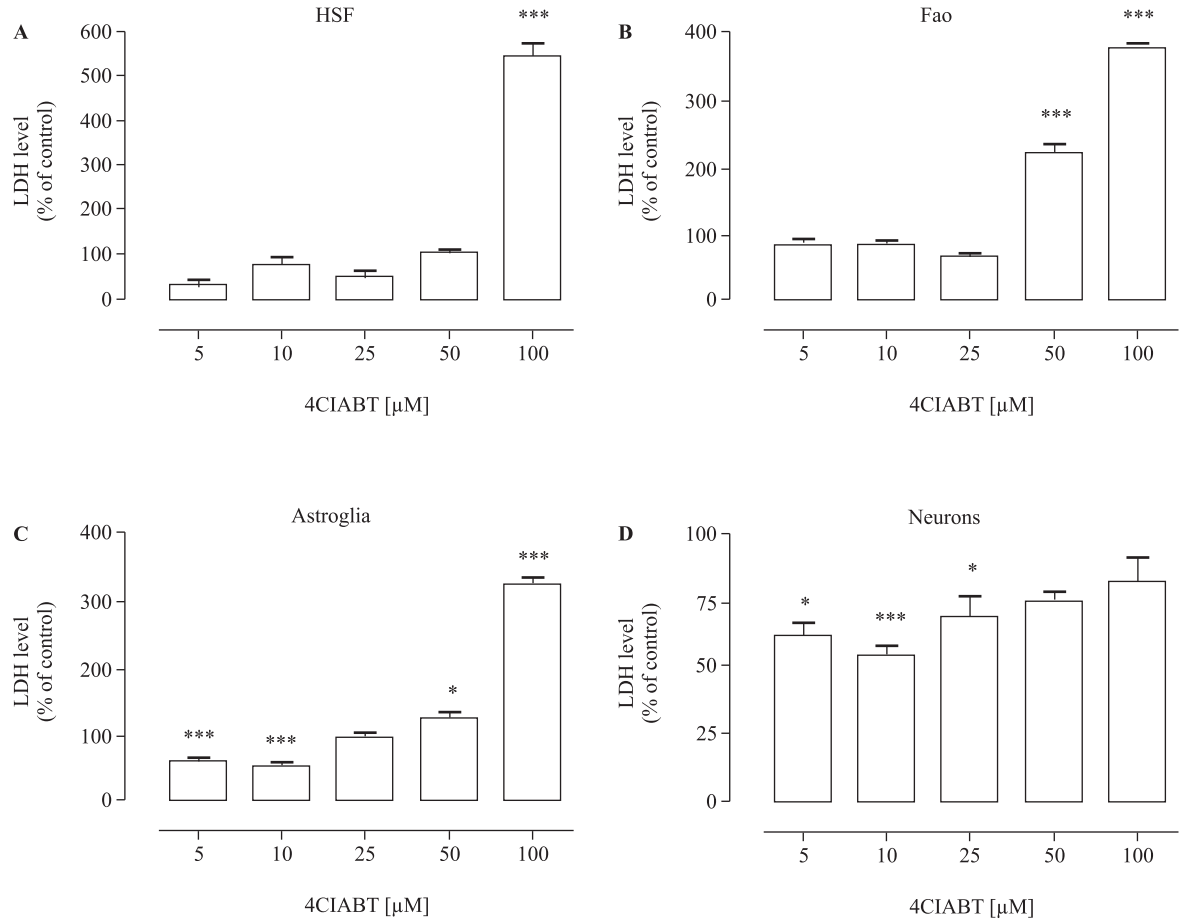

Figure 4. Cytotoxicity of 4ClABT in normal human skin fibroblast HSF (**A**), rat heptocytes Fao (**B**), rat astroglia (**C**) and mouse neurons (**D**). Cell death in the absence (control) and in the presence of 4ClABT (5–100 μM) was quantified after 24 h (HSF, Fao, astrocytes) and 48 h (neurons) treatment by measuring LDH release. Data represents mean normalized optical densities ± SEM of 4–6 trials, ***at least $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs. control, One-way ANOVA test, *post-hoc* Tukey

HE method. The studied derivative at a concentration of 5–25 μM induced changes in the morphology of both types of cancer cells, but not in normal cells. In thyroid FTC 238 cells, 4ClABT produced concentration-dependent cytoplasm shrinkage and an elongated shape of the cells (Figure 5B). Morphological changes were more prominent in T47D cells (Figure 5A). We observed a significant cytoplasm shrinkage, intensified with increased 4ClABT concentration, with almost complete cell damage at the maximum tested concentration of 25 μM. However, no toxic effect of the studied compound was observed in the normal skin fibroblast culture (Figure 5C).

Discussion

At present, cancerous diseases are one of the most frequent causes of death worldwide. Despite extensive developments in medicine and the biomedical sciences, cancer treatment remains a serious problem. Chemotherapy is usually very aggressive and induces many side-effects. Therefore, the primary aim of contemporary science and medicine is to develop a method of treatment with an effective anti-cancer action which has a low toxicity for normal tissues.

The objects of our scientific interest are aminothiadiazoles as potential anti-cancer substances. It is known that their activity and mechanism of action depend on the type of modification of the thiadiazole ring, which is pharmacophore [1, 28]. The anti-cancer activity of different derivatives has been documented in numerous publications [1, 2, 7–13]. Some of the derivatives were under clinical phase II studies, but reveal a weak ability for tumor growth inhibition, and have strong side effects [14–18]. Our 2-amino-1,3,4-thiadiazole derivatives are a very attractive option among other anti-cancer agents. We have documented the strong antiproliferative activity of 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-aminothiadiazole derivative, concomitant with relatively low toxicity.

We have reported here the action of the 2-(4-chlorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thia-

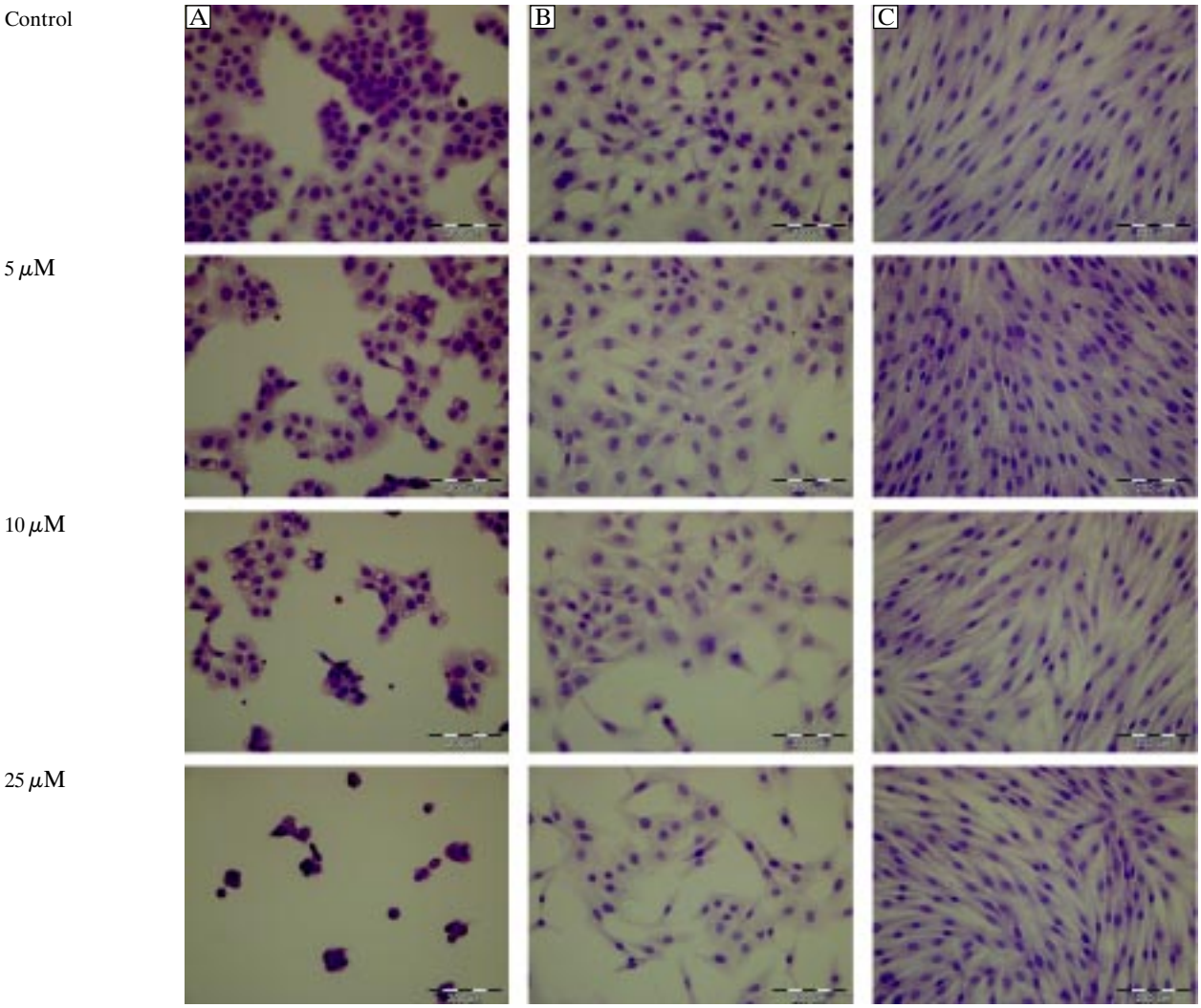

Figure 5. Effect of 4ClABT on breast carcinoma T47D cells (**A**), thyroid carcinoma FTC238 cells (**B**) and normal skin fibroblasts HSF (**C**) morphology after 48 h treatment. Micrographs show cells morphology in control culture and exposure to 5 μM, 10 μM and 25 μM 4ClABT; All images at magnification × 100

diazole (4ClABT) in cancer and normal cells, and the ability of the tested compound to inhibit proliferation of different types of cancer cells. It is significant that cancers of the nervous system (glioma, rhabdomyosarcoma/medulloblastoma, astrocytoma) were more resistant to the action of 4ClABT than cells derived from peripheral cancers. The obtained results correlate with the previously described activity of 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (FABT) [28], 2-(3-fluorophenylamino)-5-(2,4--dihydroxyphenyl)-1,3,4-thiadiazole (3FABT), and 2-(4-bromophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4--thiadiazole (4BrABT) [29]. Their action is similar, but the intensity of activity seems to depend on the atom bound to the amino-group. In the screening studies, chloro-derivatives showed the most interesting anti-cancer properties [24]. Moreover, the proved inhibition of DNA synthesis by 4ClABT confirms the ability described by Nelson et al. [10] of 2-amino-1,3,4-thiadiazole to decrease inosine 5'-phosphate (IMP) dehydrogenase activity. IMP dehydrogenase is the key enzyme in guanine nucleotides synthesis.

The important activity of 4ClABT, described previously also for bromo- and fluoro-isomers [28, 29], is the inhibition of cell motility. The ability to migrate is an important process in the development of cancer metastasis. Thus, the discovery of this action makes 4ClABT an attractive potential anti-cancer agent.

A very interesting point is the activity of the tested compound in normal cells. The performed experiments showed low cytotoxicity of 4ClABT in normal cells including skin fibroblasts, hepatocytes, astrocytes and neurons. Additionally, a significant decrease of the LDH level in astrocytes and neurons after treatment with 4ClABT may suggest a neurotrophic ac-

tion of the tested compound. This result confirms the previously described neuroprotective activity of FABT [28]. Furthermore, morphological analysis revealed differences in the action of 4ClABT on cancer and normal cells. The tested compound, at the same time exposure and concentration, induced prominent changes in breast and thyroid cancer cells, but did not damage normal fibroblasts.

Our results indicate a high level of antiproliferative activity of 4ClABT. Taking into account its relatively low toxicity, this compound seems to be very interesting as a potential anti-cancer agent.

The obtained results indicate that aminothiadiazoles act in a specific way affecting rapidly proliferating cancer cells. Therefore, we are currently investigating the molecular mechanisms of anti-cancer activity comprising the influence of tested compounds on cell cycle regulation, signal transduction pathways and apoptosis induction. Based on promising preliminary results, the *in vivo* anti-cancer verification of tested derivatives will also be performed.

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