The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

Cytotoxic effect of organochalcogen compounds against tumor cell lines

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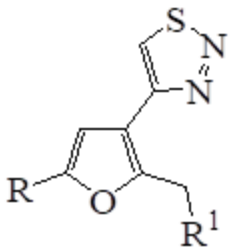
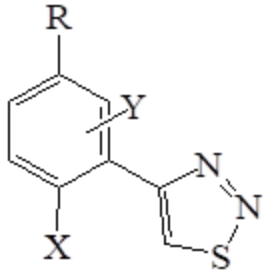
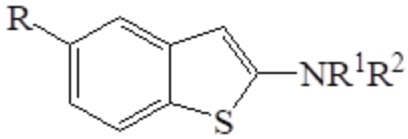
Cytotoxic effect of organochalcogen compounds against tumor cell lines



IC_{50} up to 3 μg/mL



IC_{50} up to 4 μg/mL



IC_{50} up to 9 μg/mL

Abstract: It is known that 1,2,3-thiadiazoles and their derivatives – benzo[*b*]thiophenes – have a high synthetic potential as well as a wide spectrum of biological activity. In particular, 1,2,3-thiadiazole derivatives exhibit antivirus, antitumor, herbicide, fungicide, and insecticide activity. Among the beneficial properties found for benzo[*b*]thiophene derivatives are anticancer, antidiabetic, antituberculosis, antimalarial, antifungal, antidepressant, anticonvulsant, antihyperglycemic, antiangiogenic, antimitotic, antiinflammatory and analgesic properties. The above information on 1,2,3-thiadiazole and benzo[*b*]thiophene derivatives encouraged us to design and synthesize new bioactive agents with the 4-(2-hydroxyaryl)-1,2,3-thidiazole, 4-(1,2,3-thiadiazol-4-yl)furan and 2-aminobenzo[*b*]thiophene structures. The synthesized products were tested in vitro against human erythroleukemia (K562), cervical carcinoma (HeLa), breast cancer (MCF7), T lymphocyte (Jurkat) cell lines. According to the results of the research, some derivatives showed potent antiproliferative activity against used cell lines. Obtained experimental data can stimulate further search for pharmacologically active compounds among 4-(2-hydroxyaryl)-1,2,3-thidiazole, 4-(1,2,3-thiadiazol-4-yl)furan and 2-aminobenzo[*b*]thiophene derivatives.

Keywords: antiproliferative activity; tumor cell lines; cell death; morphological changes (cytoskeleton).

Introduction

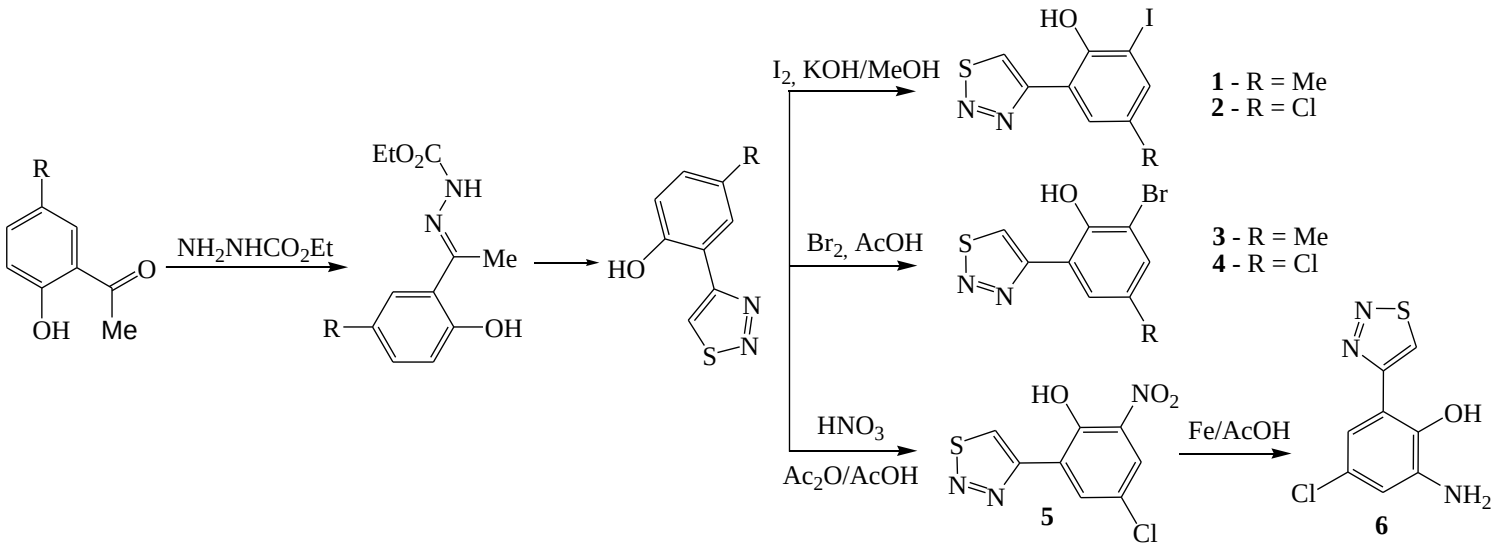
Oncological diseases are one of the most common public health problems and the second leading cause of death after cardiovascular disease. Increased drug resistance and the emergence of tumor resistance as well as severe side-effects of chemotherapeutic agents reduce the clinical efficacy of currently used anticancer drugs and treatments. Despite the increasing use of targeted drugs and methods of immunotherapy of oncological diseases, the development of cytostatic agents remains an important challenge for the treatment of cancer. At the same time, the emergence of tumor resistance requires the creation of cytostatics that are not just derivatives of "classical" drugs, but originating from compounds of a new nature.

1,2,3-Thiadiazoles and their derivatives – benzo[*b*]thiophenes – have a high synthetic potential as well as a wide spectrum of biological activity. In particular, 1,2,3-thiadiazole derivatives exhibit antivirus, antitumor, herbicide, fungicide, and insecticide activity. Among the beneficial properties found for benzo[*b*]thiophene derivatives are anticancer, antidiabetic, antituberculosis, antimalarial, antifungal, antidepressant, anticonvulsant, antihyperglycemic, antiangiogenic, antimitotic, antiinflammatory and analgesic properties. This encouraged us to design and synthesize new bioactive agents with the 4-(2-hydroxyaryl)-1,2,3-thidiazole, 4-(1,2,3-thiadiazol-4-yl)furan and 2-aminobenzo[*b*]thiophene structures. All the compounds were evaluated for their antiproliferative activity as well as morphological changes and cell death were evaluated for the most active products.

Results and discussion

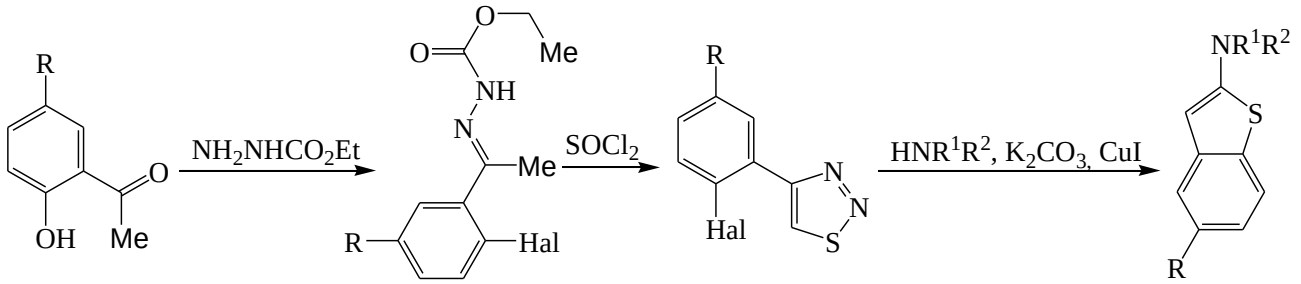
Synthesis

The synthesis of substituted 4-aryl- 1,2,3-thidiazoles was based on the Hurd-Mori reaction which led to 1,2,3-thiadiazoles 3a and 3b. The following reaction of 4-aryl-1,2,3-thiadiazoles 3a, 3b with iodine in alkali media (KOH) at RT gave iodides **1** and **2** with high yields, while reaction with bromine in acetic acid media gave corresponding bromides **3** and **4**. The nitro derivative **5** was obtained under the action of the nitrating mixture – HNO_3 in Ac_2O/AcOH.



Results and discussion

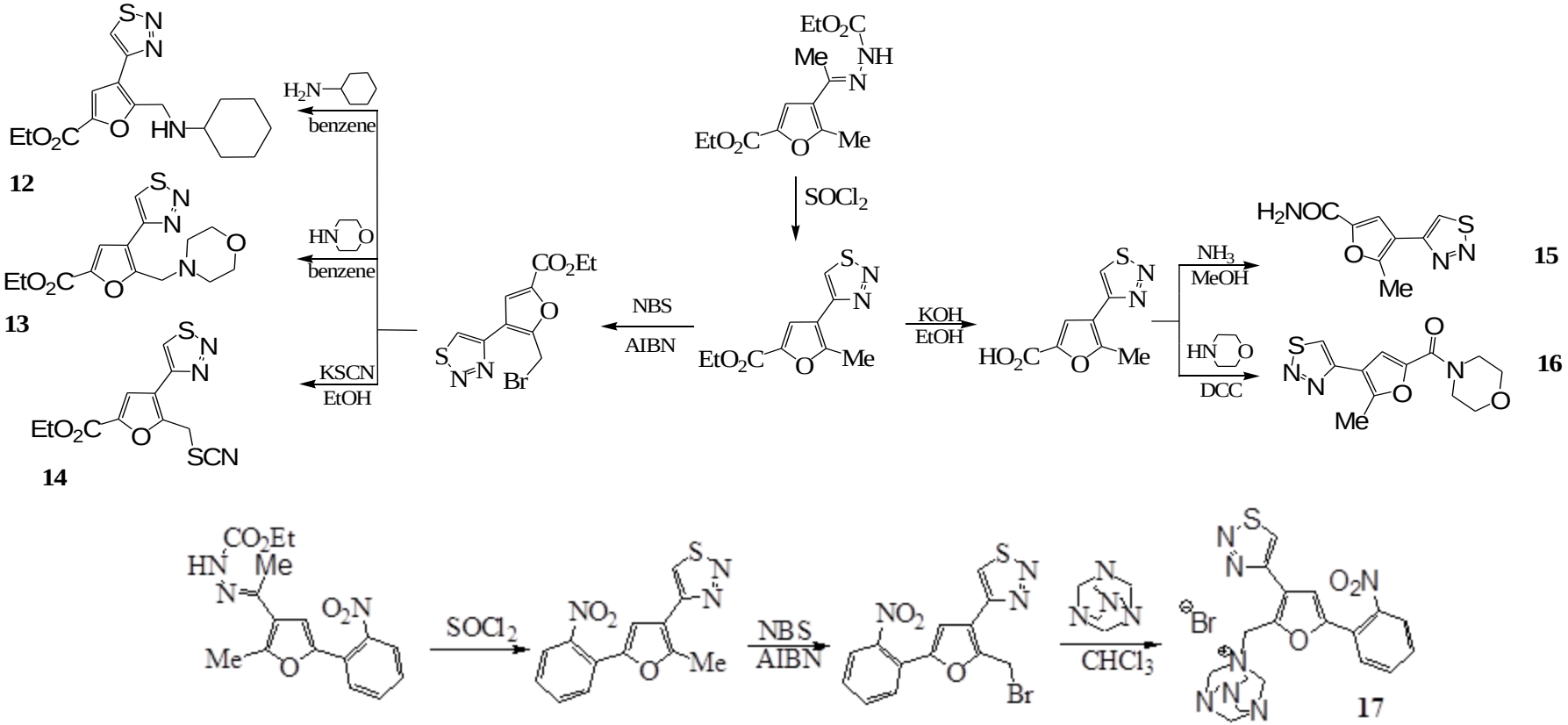
2-Aminobenzo[*b*]thiophenes **7-10** were synthesized by reaction of corresponding 1,2,3-thiadiazole with various secondary amines such as pyrrolidine, 2-ethylpiperidine and morpholine in DMF in the presence of copper(I) iodide and potassium carbonate. 4-(4-Nitrobenzo[*b*]-thiophen-2-yl)morpholine (**11**) was obtained by reaction of corresponding 1,2,3-thiadiazole with morpholine in DMF medium without a catalyst.



7 - R = H, NR^1R^2 = pyrrolidin-1-yl;
8 - R = H, NR^1R^2 = 2-ethylpiperidin-1-yl;
9 - R = H, NR^1R^2 = morpholin-4-yl;
10 - R = MeO, NR^1R^2 = morpholin-4-yl;
11 - R = NO_2, NR^1R^2 = morpholin-4-yl

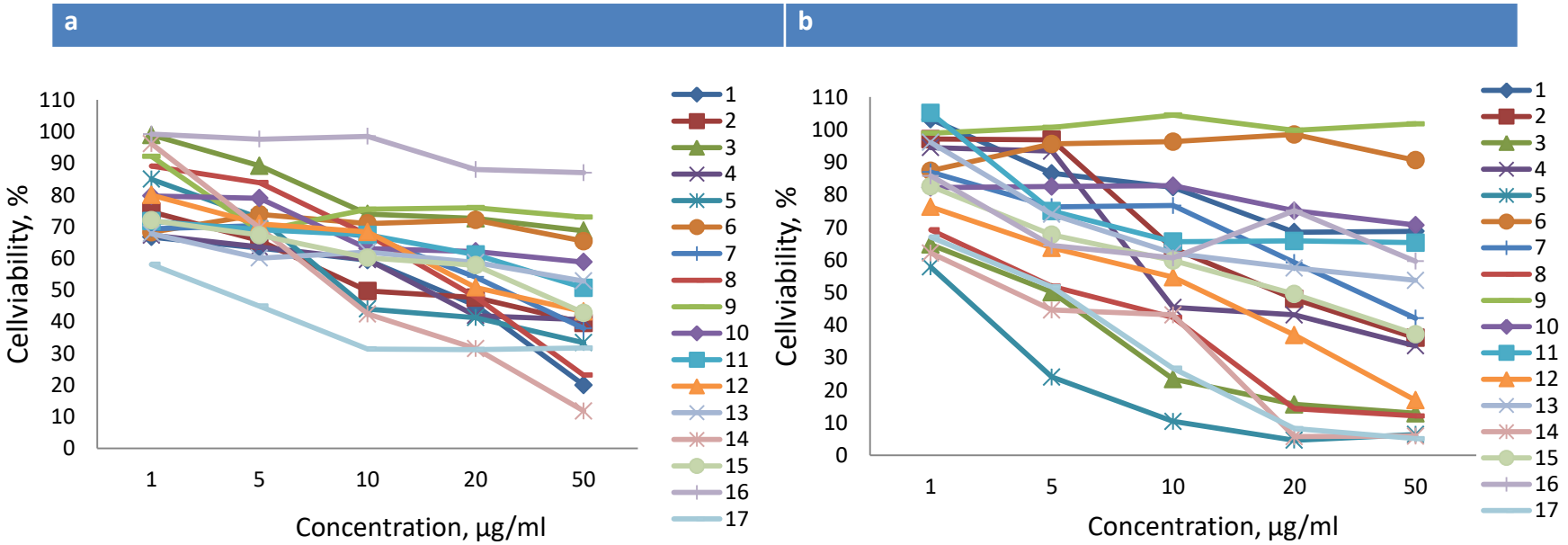
Results and discussion

Synthesis of 4-furyl-1,2,3-thiadiazoles **12 – 17** is outlined at followed Scheme



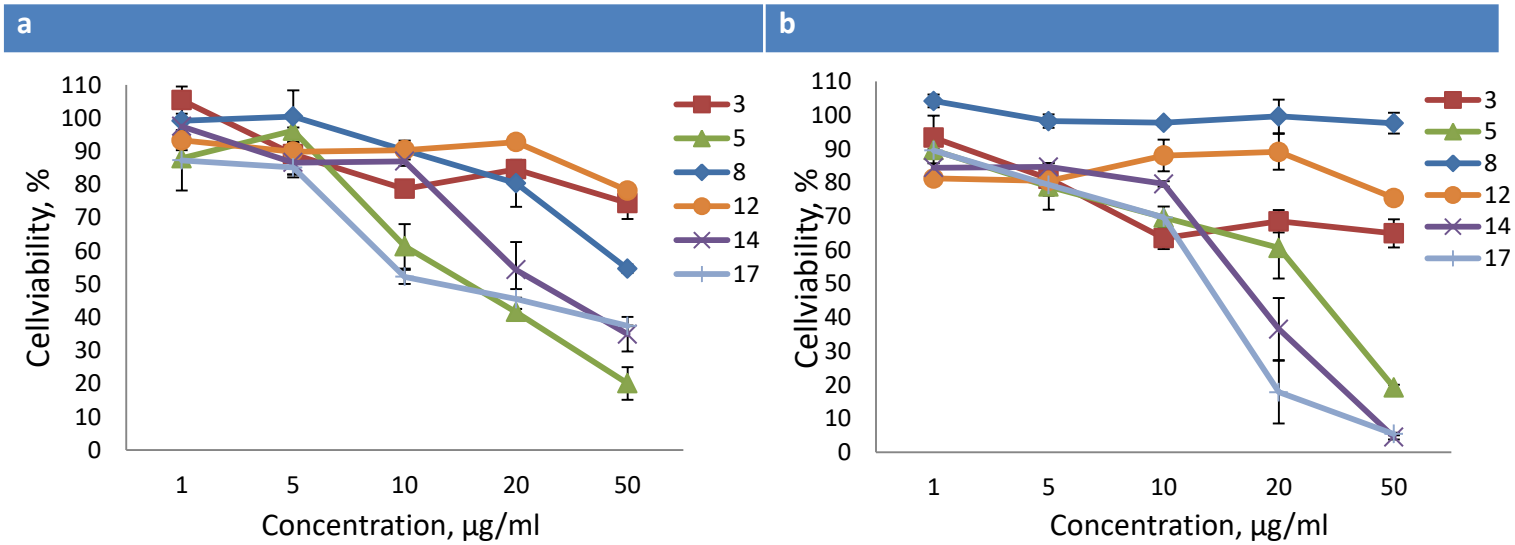
Antiproliferative effect of synthesized compounds against cancer cell lines

Antiproliferative activity of synthesized derivatives **1 – 17** against human cervical carcinoma (HeLa), erythroleukemia (K562), breast cancer (MCF7) and T lymphocyte (Jurkat) cell line was evaluated *in vitro* by the standard MTS assay for 24 and 72 h.



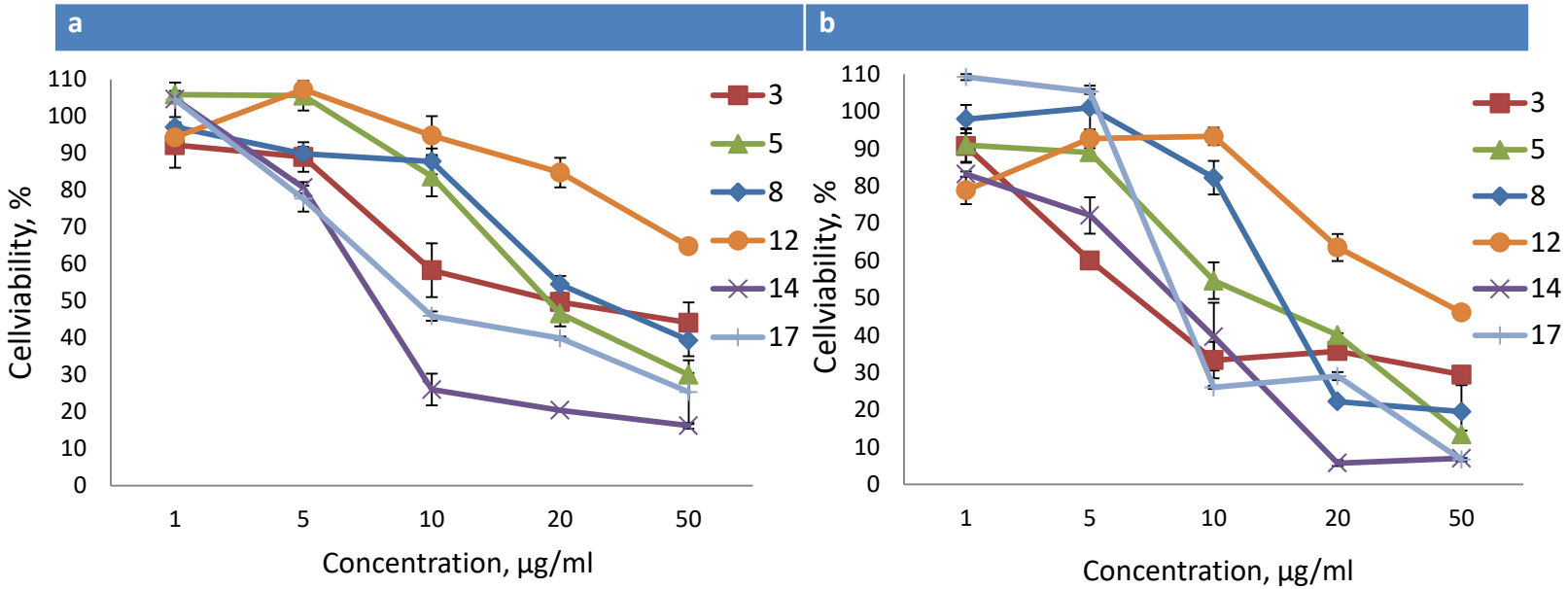
Antiproliferative activity of 4-(2-hydroxyaryl)-1,2,3-thidiazoles, 4-(1,2,3-thiadiazol-4-yl)furanes and 2-aminobenzo[b]thiophenes against K562 cell line for 24 (a) and 72 (b) h.

Antiproliferative effect of synthesized compounds against cancer cell lines



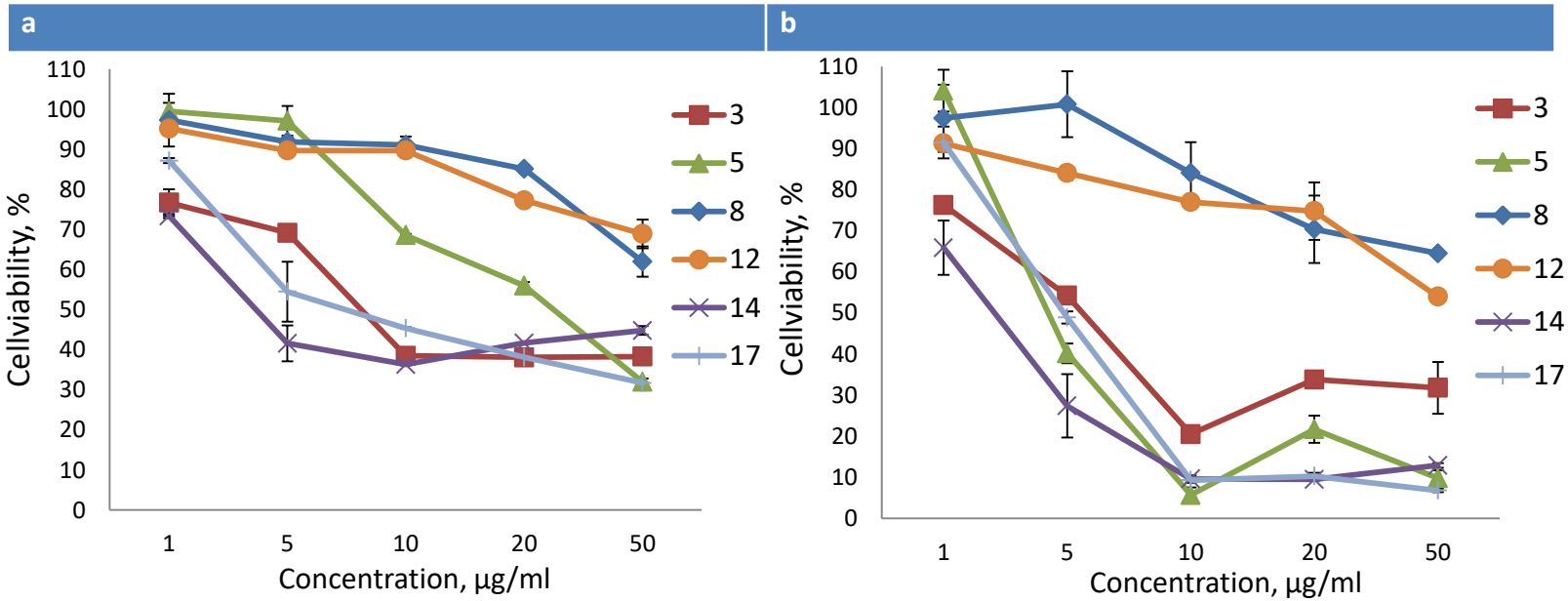
Antiproliferative activity of 4-(2-hydroxyaryl)-1,2,3-thidiazoles, 4-(1,2,3-thiadiazol-4-yl)furanes and 2-aminobenzo[b]thiophenes against HeLa cell line for 24 (a) and 72 (b) h.

Antiproliferative effect of synthesized compounds against cancer cell lines



Antiproliferative activity of 4-(2-hydroxyaryl)-1,2,3-thidiazoles, 4-(1,2,3-thiadiazol-4-yl)furanes and 2-aminobenzo[b]thiophenes against MCF7 cell line for 24 (a) and 72 (b) h.

Antiproliferative effect of synthesized compounds against cancer cell lines



Antiproliferative activity of 4-(2-hydroxyaryl)-1,2,3-thidiazoles, 4-(1,2,3-thiadiazol-4-yl)furanes and 2-aminobenzo[b]thiophenes against Jurkat cell line for 24 (a) and 72 (b) h.

Antiproliferative effect of synthesized compounds against cancer cell lines

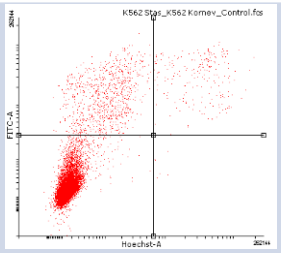
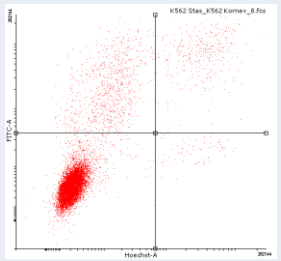
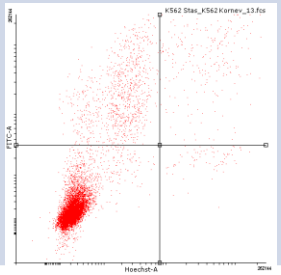
Соединение	IC_{50}, μg/mL							
	K562		HeLa		MCF7		Jurkat	
	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
8	29±5	9±1			27 ±2	16±1		
3	30±6	7±1			20 ±1	7±1	8±1	4±1
5	12±3	5±1	15 ±4	25 ±4	19 ±1	13±1	17±1	4±1
14	12±3	4±1	25 ±7	18 ±1	8±1	8±1	4±1	3±1
12	43±3	12±1		-	-	40±3	-	-
17	9±1	8±1	12 ±2	15 ±2	9±1	8±1	7±3	5±1

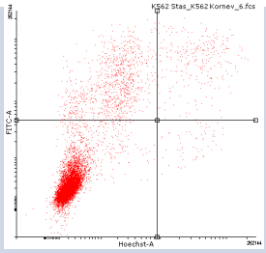
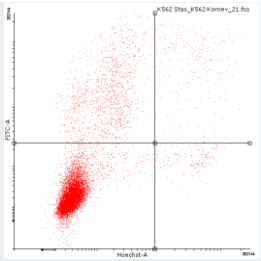
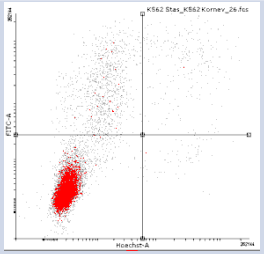
IC_{50} values of most active 4-(2-hydroxyaryl)-1,2,3-thidiazoles, 4-(1,2,3-thiadiazol-4-yl)furanes and 2-aminobenzo[b]thiophenes

Cell death analysis

The apoptotic effect of 4-(2-hydroxyaryl)-1,2,3-thidiazoles **3** and **5**, 4-furyl-1,2,3-thiadiazoles **12** and **14,** 2-aminobenzo[b]thiophene **8** was evaluated by Annexin V-FITC/propidium iodide (AV/PI) dual staining assay to examine the occurrence of phosphatidylserine externalization, which facilitated the detection of live cells (lower left quadrant; AV-/PI-), early apoptotic cells (upper left quadrant; AV+/PI-), late apoptotic cells (upper right quadrant; AV+/PI+) and necrotic cells (lower right quadrant; AV-/PI+).

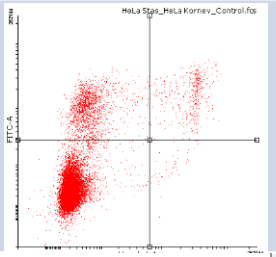
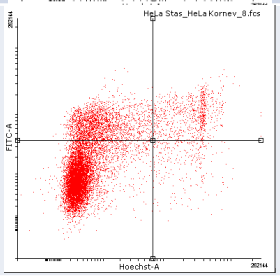
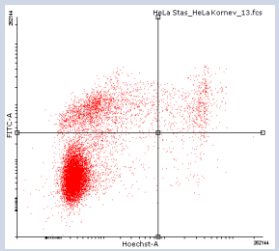
Cell death analysis

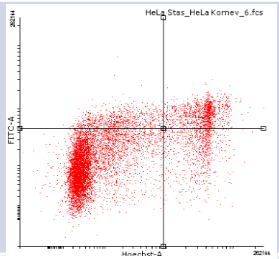
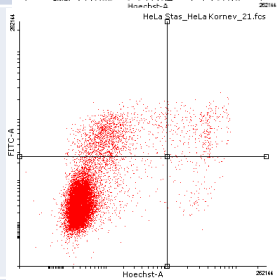
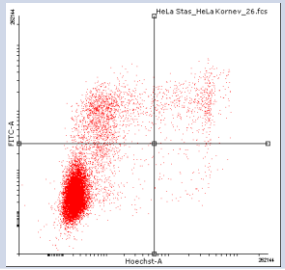
K562		
Control		Live: 90.46 Early apoptosis: 7.56 Late apoptosis: 1.92
3		Live: 89.61 Early apoptosis: 7.03 Late apoptosis: 2.79
5		Live: 90.83 Early apoptosis: 6.77 Late apoptosis: 1.82

K562		
8		Live: 89.11 Early apoptosis: 8.0 Late apoptosis: 2.37
14		Live: 87.17 Early apoptosis: 9.91 Late apoptosis: 1.67
17		Live: 88.64 Early apoptosis: 8.73 Late apoptosis: 2.12

Annexin V-FITC/Propidium iodide (PI) dual staining assay of **K562** cells treated with compounds **3**, **5**, **8**, **12** and **14** at concentrations 10 μg/mL using flow cytometry

Cell death analysis

HeLa		
Control		Live: 85.23 Early apoptosis: 11.36 Late apoptosis: 3.13
3		Live: 71.95 Early apoptosis: 19.16 Late apoptosis: 6.66
5		Live: 79.08 Early apoptosis: 15.73 Late apoptosis: 4.36

K562		
8		Live: 70.67 Early apoptosis: 8.09 Late apoptosis: 12.84
14		Live: 87.67 Early apoptosis: 9.36 Late apoptosis: 2.22
17		Live: 85.27 Early apoptosis: 10.33 Late apoptosis: 3.78

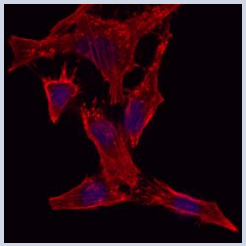
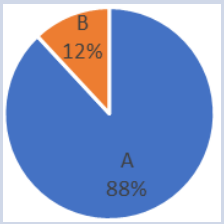
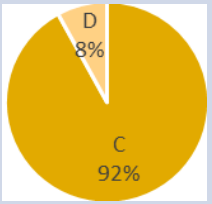
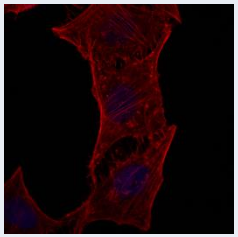
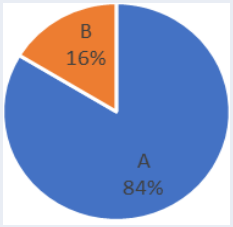
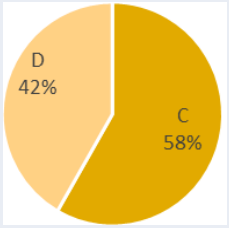
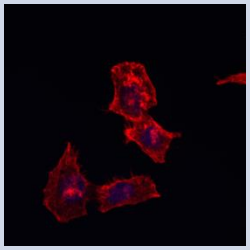
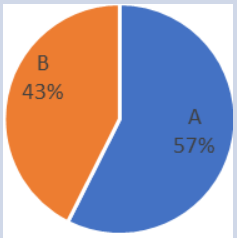
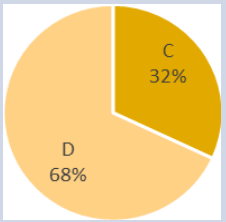
Annexin V-FITC/Propidium iodide (PI) dual staining assay of **HeLa** cells treated with compounds **3**, **5**, **8**, **12** and **14** at concentrations 10 μg/mL using flow cytometry

Actine cytoskeleton changes

The structure of the actin cytoskeleton of HeLa and MCF7 cells was assessed by the availability of stress fibers and the presence of filopodia-like protrusions after the impact of most active compounds.

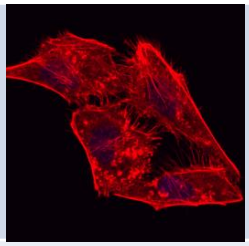
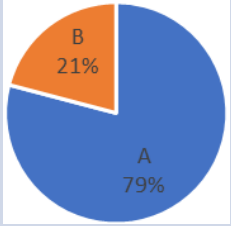
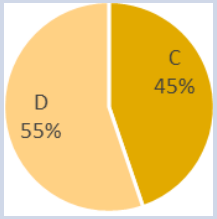
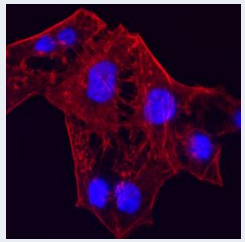
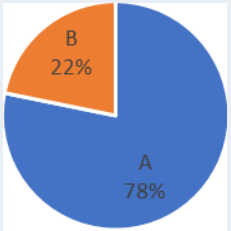
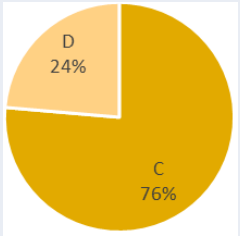
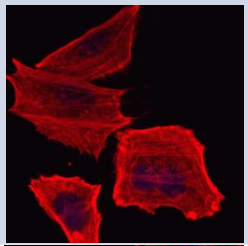
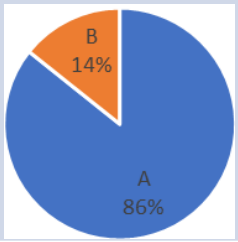
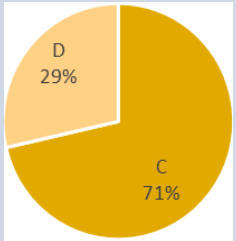
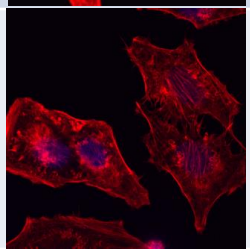
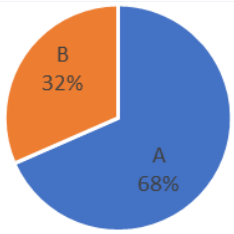
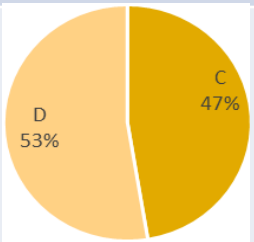
It was found using confocal microscopy that treatment with 4-(2-hydroxyaryl)-1,2,3-thidiazoles, 4-(1,2,3-thiadiazol-4-yl)furanes and 2-aminobenzo[b]thiophenes led to significant changes of the actin cytoskeleton structure of tumor cells leading to the disappearance of stress fibers and changes in the number of filopodia-like deformations.

Actine cytoskeleton changes

Sample	I	II	III
Hela control			
3			
5			

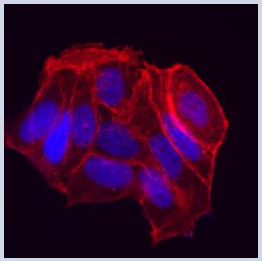
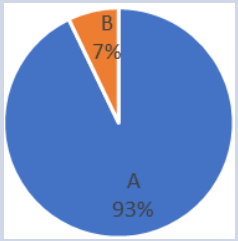
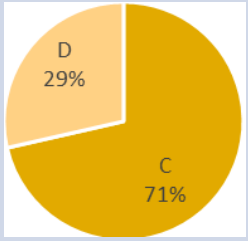
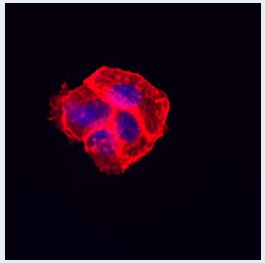
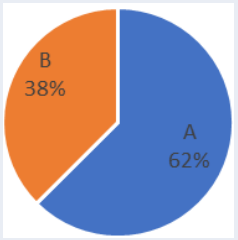
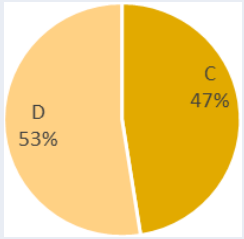
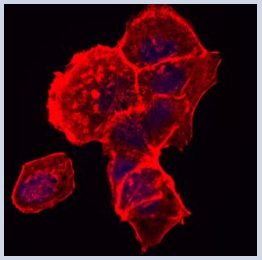
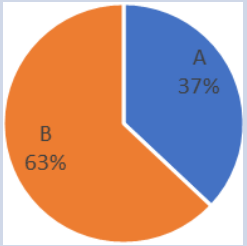
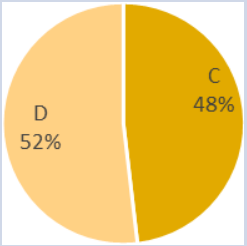
State of actin cytoskeleton of **HeLa** cells after treatment with compounds **3**, **5**, **8**, **12**, **14, 17**.
I: Images demonstrate the different stages of cell actin cytoskeleton.
II: Pie charts demonstrate percentage of cells with normal stress fibers (A) and disassembled stress fibers (B).
III: Pie charts demonstrate percentage of cells with filopodia-like deformations (C), and without filopodia-like deformations (D).

Actine cytoskeleton changes

Sample	I	II	III
8			
12			
14			
17			

State of actin cytoskeleton of **HeLa** cells after treatment with compounds **3**, **5**, **8**, **12**, **14, 17**.
I: Images demonstrate the different stages of cell actin cytoskeleton.
II: Pie charts demonstrate percentage of cells with normal stress fibers (A) and disassembled stress fibers (B).
III: Pie charts demonstrate percentage of cells with filopodia-like deformations (C), and without filopodia-like deformations (D).

Actine cytoskeleton changes

Sample	I	II	III
Control			
3			
5			

State of actin cytoskeleton of **MCF7** cells after treatment with compounds **3**, **5**, **8**, **12**, **14, 17**.
I: Images demonstrate the different stages of cell actin cytoskeleton.
II: Pie charts demonstrate percentage of cells with normal stress fibers (A) and disassembled stress fibers (B).
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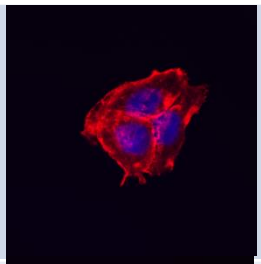
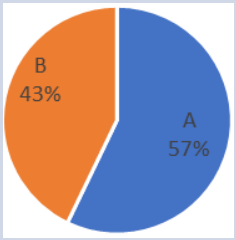
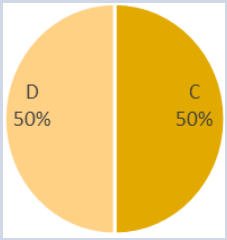
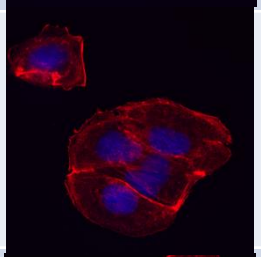
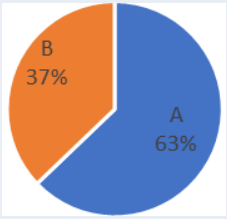
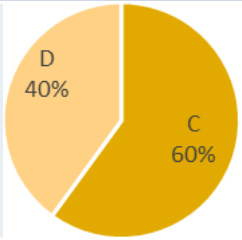
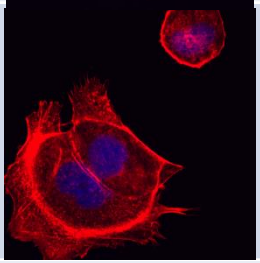
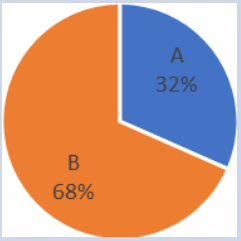
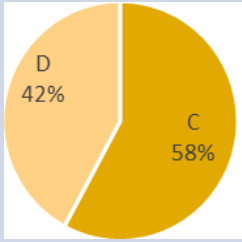
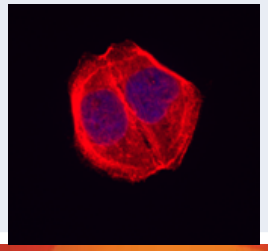
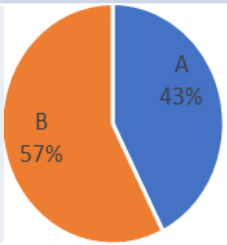
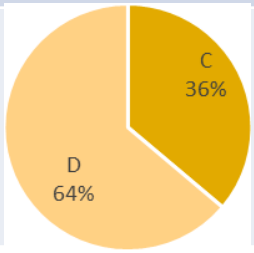
Actine cytoskeleton changes

Sample	I	II	III
8			
12			
14			
17			

State of actin cytoskeleton of **MCF7** cells after treatment with compounds **3**, **5**, **8**, **12**, **14, 17**.
I: Images demonstrate the different stages of cell actin cytoskeleton.
II: Pie charts demonstrate percentage of cells with normal stress fibers (A) and disassembled stress fibers (B).
III: Pie charts demonstrate percentage of cells with filopodia-like deformations (C), and without filopodia-like deformations (D).

Conclusions

In this study, we have established the ability of heterocyclic compounds containing 4-(1,2,3-thia-diazol-4-yl)furane, 4-(2-hydroxyaryl)-1,2,3-thidiazole and 2-aminobenzo[*b*]thiophene framework to reduce the viability of human erythroleukemia (K562), human cervical carcinoma (HeLa), breast cancer (MCF7) and T lymphocyte (Jurkat) cell lines. K562 cell line was the most sensitive against the screened cell lines. Among tested compounds 4-(1,2,3-thia-diazol-4-yl)furanes were more active against all used cell lines. Screened compounds were shown to downregulate growth of Hela cells, as well as lead to a decrease in the number of cells with normal stress fibers and filopodia-like deformations.

Obtained data make it possible to assume that 4-(1,2,3-thia-diazol-4-yl)furane, 4-(2-hydroxyaryl)-1,2,3-thidiazole and 2-aminobenzo[*b*]thiophene framework may be considered as promising pharmacophore units for further screenings.

Acknowledgments

This research was financially supported by the grant of the Russian Science Foundation, RSF 20-15-00332.