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Study of cytotoxicity of spiro-fused [3-azabicyclo[3.1.0]hexane]oxindoles against tumor cell lines

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pharmaceuticals MDPI

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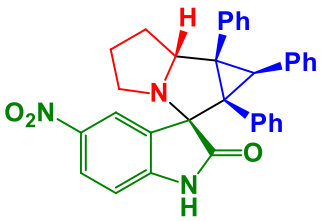
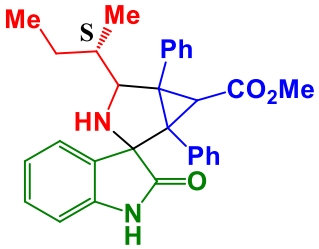
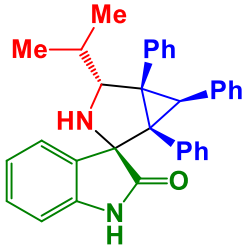
Study of cytotoxicity of spiro-fused [3-azabicyclo[3.1.0]hexane]oxindoles against tumor cell lines



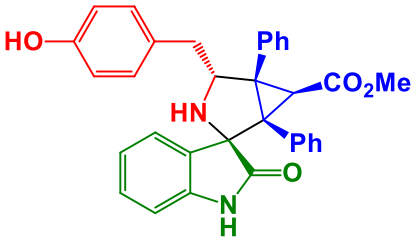
2, IC_{50} up to 3 μg/mL



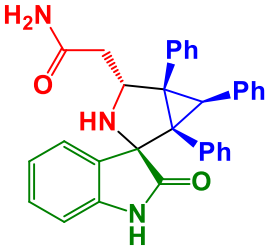
4, IC_{50} up to 3 μg/mL



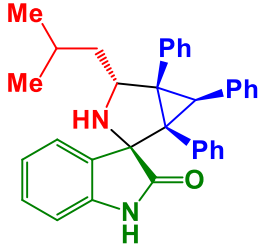
8, IC_{50} up to 3 μg/mL



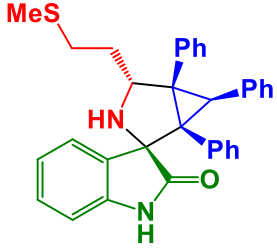
15, IC_{50} up to 8 μg/mL



18, IC_{50} up to 2 μg/mL



24, IC_{50} up to 4 μg/mL



26, IC_{50} up to 3 μg/mL

Abstract: A series of heterocyclic compounds containing 3-spiro[3-azabicyclo[3.1.0]-hexane]oxindole framework have been studied for their antiproliferative activity against human erythroleukemia (K562), T lymphocyte (Jurkat), cervical carcinoma (HeLa) and breast cancer (MCF-7) as well as mouse colon carcinoma (CT26) cell lines. This spiro-fused adducts are readily available *via* one-pot three-component 1,3-dipolar cycloaddition reactions of cyclopropenes and azomethine ylides (generated from oxindoles and amino acids *in situ*). It was found that some of these compounds significantly reduce proliferative activity at concentrations less that 10 μg/mL. Additionaly they achieved significant cell-cycle perturbation with higher accumulation of cells in G0/G1 phase. Also we discovered that actin filaments disappeared and granular actin was distributed diffusely in the cytoplasm in up to 38% of treated cells while the number of cells with filopodium-like membrane protrusions was significantly reduced after treatment with some of tested compounds (from 93 % in control cells up to 64% after treatment), which indirectly suggests a decrease in cell motility. The obtained results support the antitumor effect of the studied compounds and encourage the extension of the study in order to improve the anticancer activity and reduce the toxicological risks of the obtained compounds.

Keywords: antiproliferative activity; cell cycle; morphological changes (cytoskeleton); spiro-fused [3-azabicyclo[3.1.0]hexane]oxindoles; tumor cell lines

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 occurence of tumor resistance as well as serious side effects of chemotherapeutic agents, reduce the clinical effectiveness of currently used antitumor drugs and treatment methods. All this requires the creation of cytostatics, which are not just derivatives of "classical" drugs, but come from compounds of a new nature.

Natural products or synthetic compounds created on the basis of natural products are still excellent sources of new candidate drugs. Many of the most currently applicable anticancer drugs are either compounds of natural origin themselves, or developed on the basis of natural compounds.

Recent advances in the synthesis of complex heterocyclic systems have led to a significant increase in interest in the development of effective methods for the synthesis of various derivatives and structural analogues of these compounds as potential drugs. Oxindole, azabicyclohexane and pyrrolizine frameworks are well-known heterocyclic motifs that form the core of a large family of alkaloid natural products with strong profiles of biological activity.

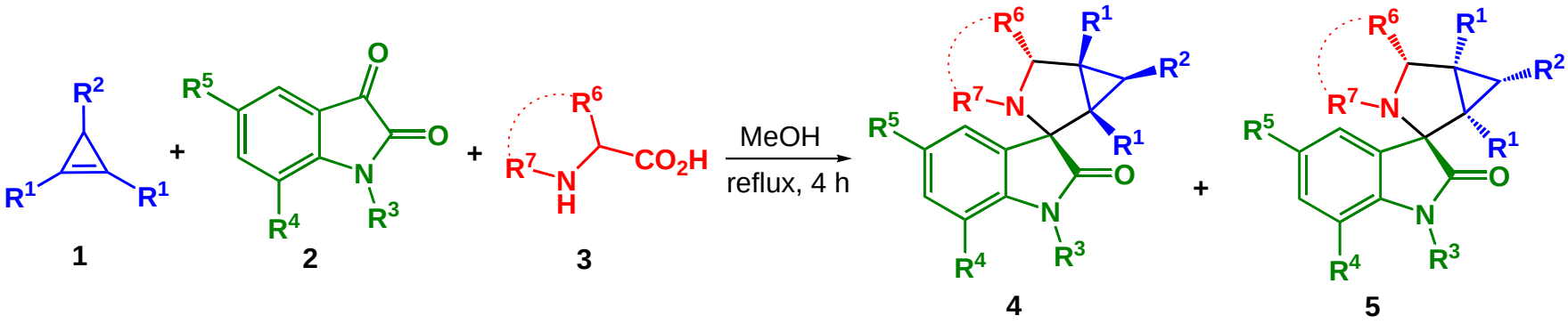
We here report on the study of spiro-fused [3-azabicyclo[3.1.0]hexane]oxindoles (readily available *via* one-pot three-component 1,3-dipolar cycloadditions of *in situ* generated isatin-derived azomethine ylides with various cyclopropenes) for their antiproliferative activity against selected cell lines as well as morphological changes and cell cycle perturbations during treatment with the most active products.

Results and discussion

Synthesis

One-pot three-component 1,3-dipolar cycloaddition reactions of various cyclopropenes with *in situ* generated isatin-derived azomethine ylides lead to the formation of desired spiroadducts either as a single isomer **4** or as two diastereomeres **4** and **5** with up to 83% overall isolation yield. The ratio of products **4** and **5** in diastereomeric mixtures was found to be at range 13-2 to 1 correspondingly. The major diastereomer in all cases was product **4**.

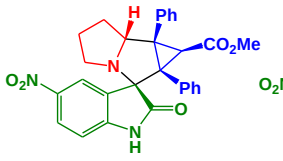
Synthesis and structure elucidation was described in detail by us earlier at JOC 2017, 82, 959-975.



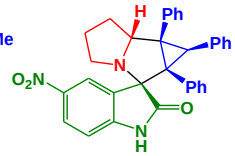
1 2 3 4 5

Results and discussion

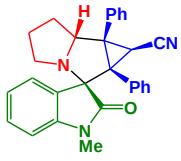
Structures of screened products



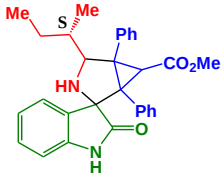
1, 58% yield
6:1 *dr*



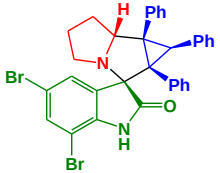
2, 60% yield
5:1 *dr*



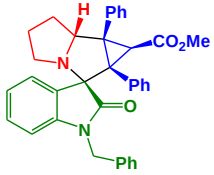
3, 83% yield
single isomer



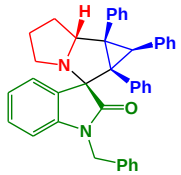
4, 57% yield
2:1 *dr*



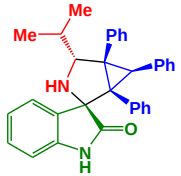
5, 44% yield
single isomer



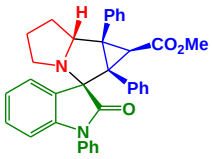
6, 80% yield
single isomer



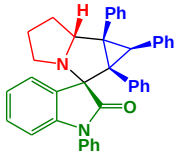
7, 77% yield
single isomer



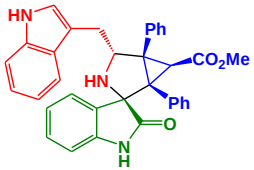
8, 44% yield
single isomer



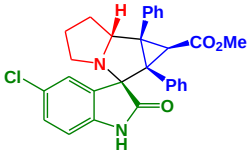
9, 65% yield
12:1 *dr*



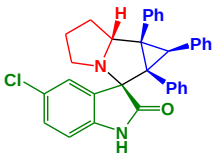
10, 62% yield
11:1 *dr*



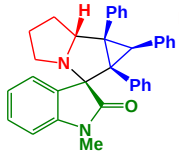
11, 48% yield
single isomer



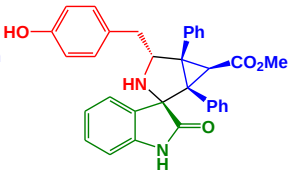
12, 63% yield
10:1 *dr*



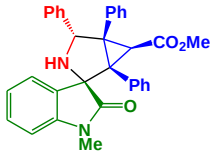
13, 56% yield
9:1 *dr*



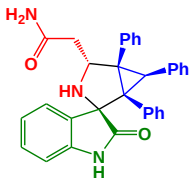
14, 72% yield
single isomer



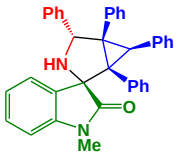
15, 59% yield
single isomer



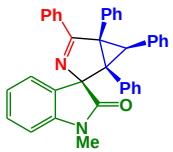
16, 79% yield
13:1 *dr*



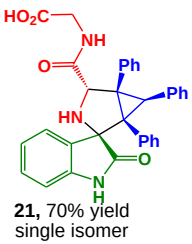
18, 45% yield
single isomer



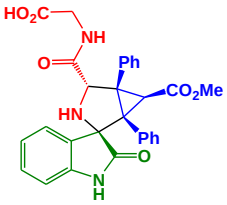
19, 87% yield
single isomer



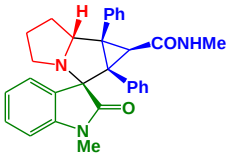
20, 61% yield
single isomer



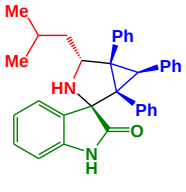
21, 70% yield
single isomer



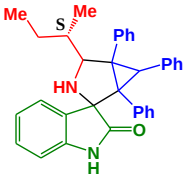
22, 57% yield
10:1 *dr*



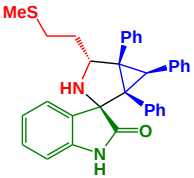
23, 55% yield
single isomer



24, 67% yield
6:1 *dr*



25, 60% yield
2:1 *dr*



26, 58% yield
single isomer

Results and discussion

Antiproliferative effect of synthesized compounds against cancer cell lines

Antiproliferative activity of synthesized spiro-fused [3-azabicyclo[3.1.0]hexane]-oxindoles was evaluated *in vitro* by the standard MTS assay for 24 and 72 h against human erythroleukemia (K562), T lymphocyte (Jurkat), cervical carcinoma (HeLa) and breast cancer (MCF-7) as well as mouse colon carcinoma (CT26) cell lines.

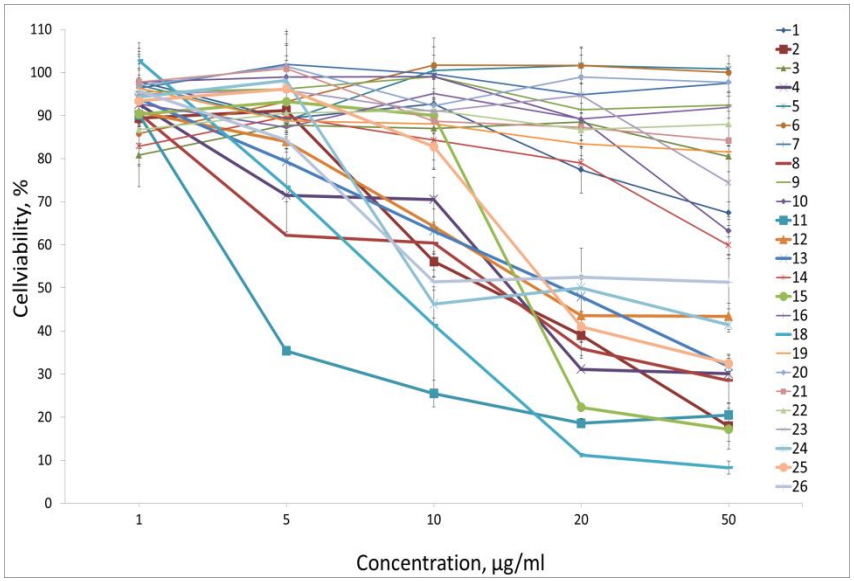
It was found that Jurkat cell line was the most sensitive to the screened compounds among the tested cell lines with IC_{50} ranging from 2±1 to 8±2 μg/mL (72 h).

It was noticed that products with unsubstituted at nitrogen atom of oxindol unit were more active against all studied cell lines. At the same time among tested products bearing three phenyl substituents at cyclopropane unit were usually more effective. All the active compounds significantly reduce proliferative activity at concentrations less that 10 μg/mL.

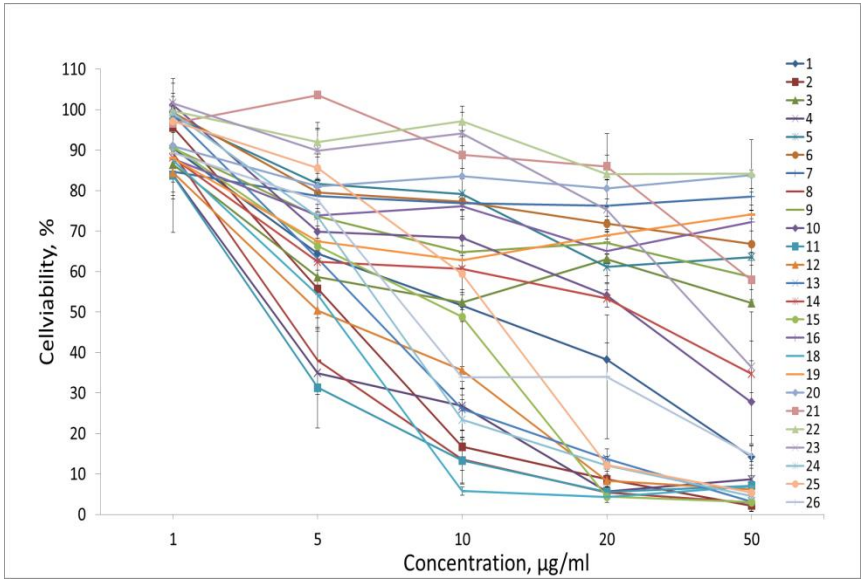
Results and discussion

Antiproliferative effect of synthesized compounds against cancer cell lines

A



B

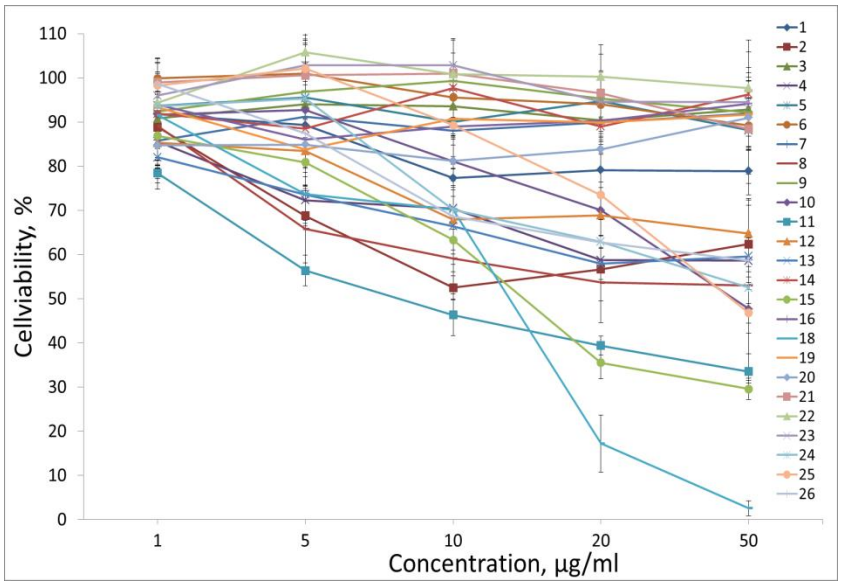


Antiproliferative activity of spiro-fused [3-azabicyclo[3.1.0]hexane]oxindoles against **K562** cell lines for 24 (A) and 72 (B) h.

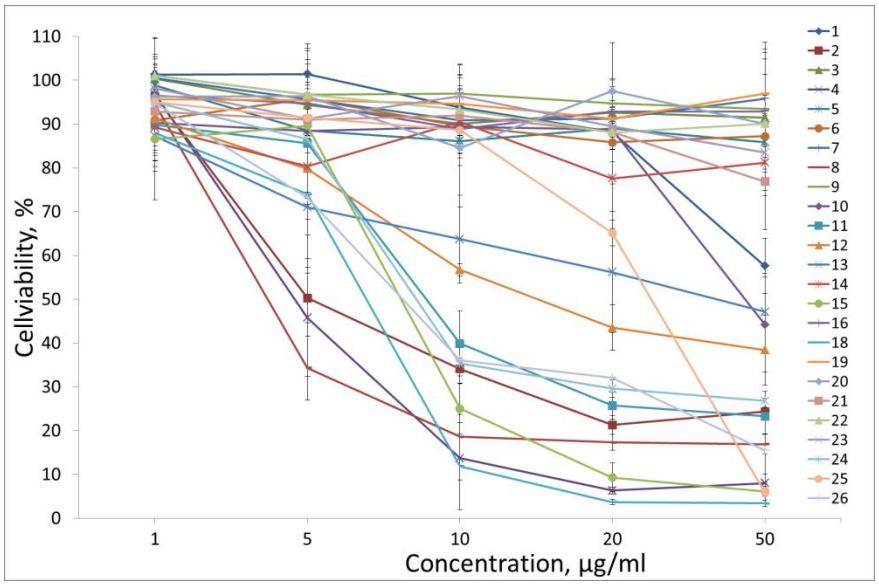
Results and discussion

Antiproliferative effect of synthesized compounds against cancer cell lines

A



B

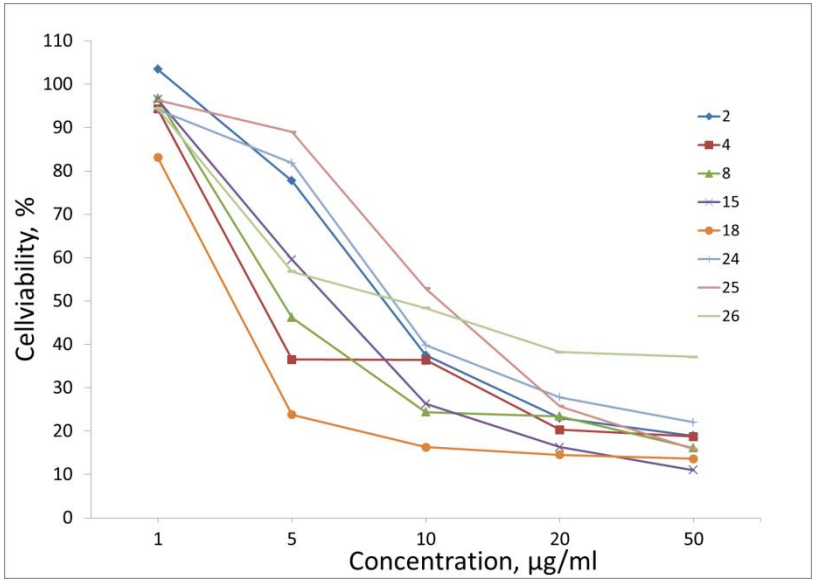


Antiproliferative activity of spiro-fused [3-azabicyclo[3.1.0]hexane]oxindoles against **HeLa** cell lines for 24 (A) and 72 (B) h.

Results and discussion

Antiproliferative effect of synthesized compounds against cancer cell lines

A



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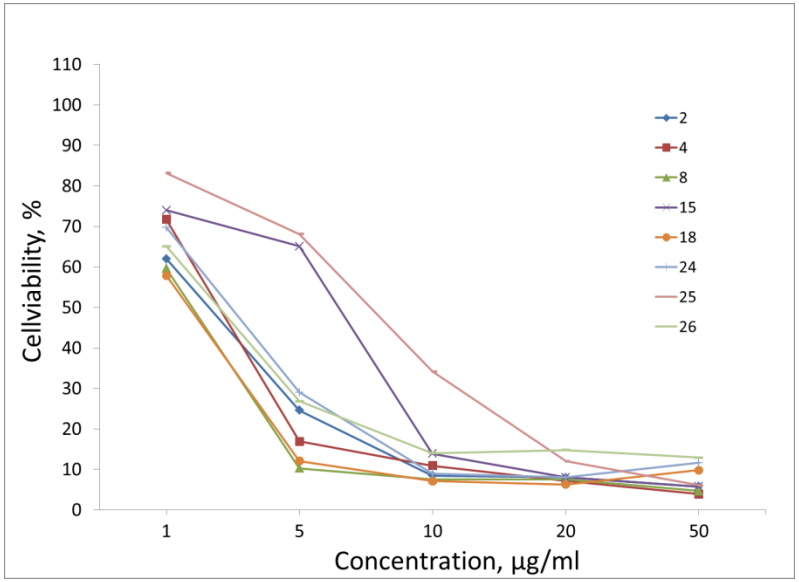
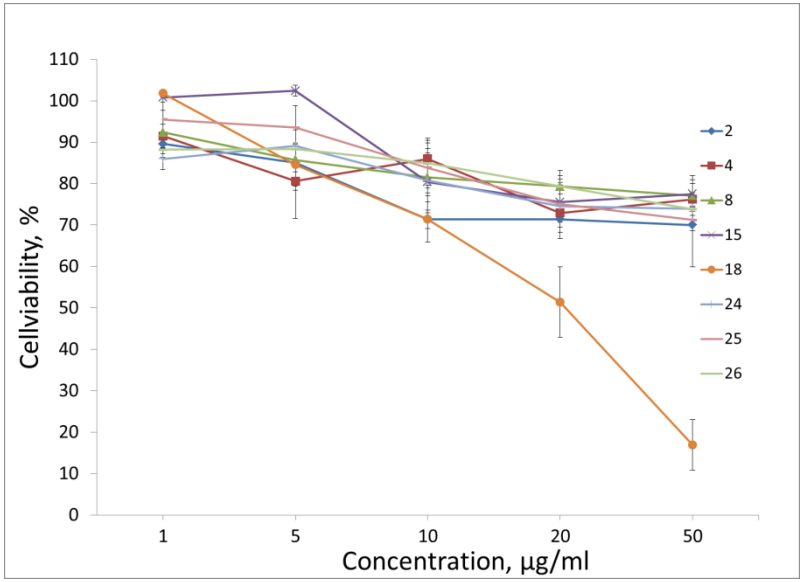


Antiproliferative activity of spiro-fused [3-azabicyclo[3.1.0]hexane]oxindoles against **Jurkat** cell lines for 24 (A) and 72 (B) h.

Results and discussion

Antiproliferative effect of synthesized compounds against cancer cell lines

A



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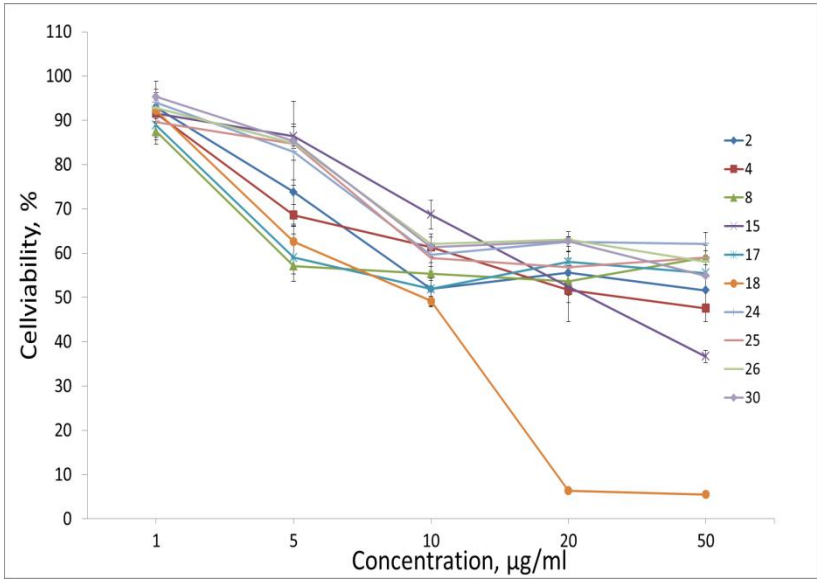
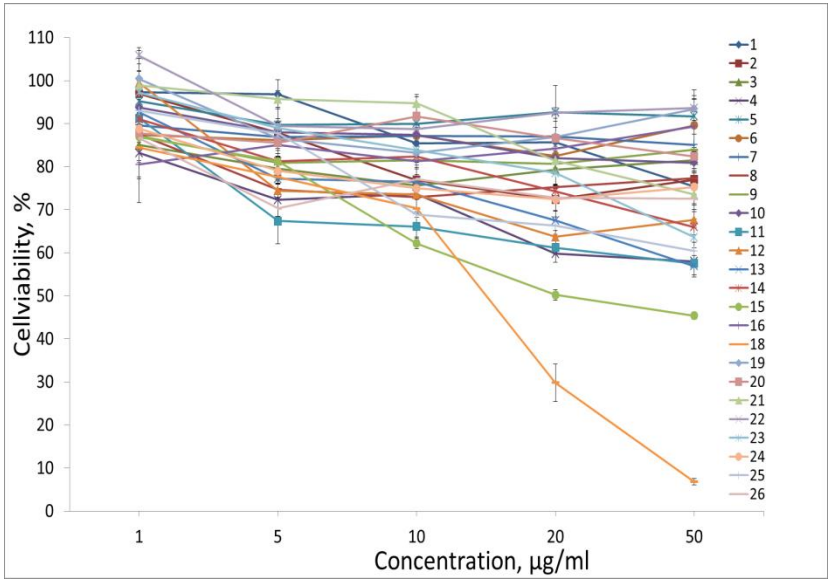


Antiproliferative activity of spiro-fused [3-azabicyclo[3.1.0]hexane]oxindoles against **MCF-7** cell lines for 24 (A) and 72 (B) h.

Results and discussion

Antiproliferative effect of synthesized compounds against cancer cell lines

A



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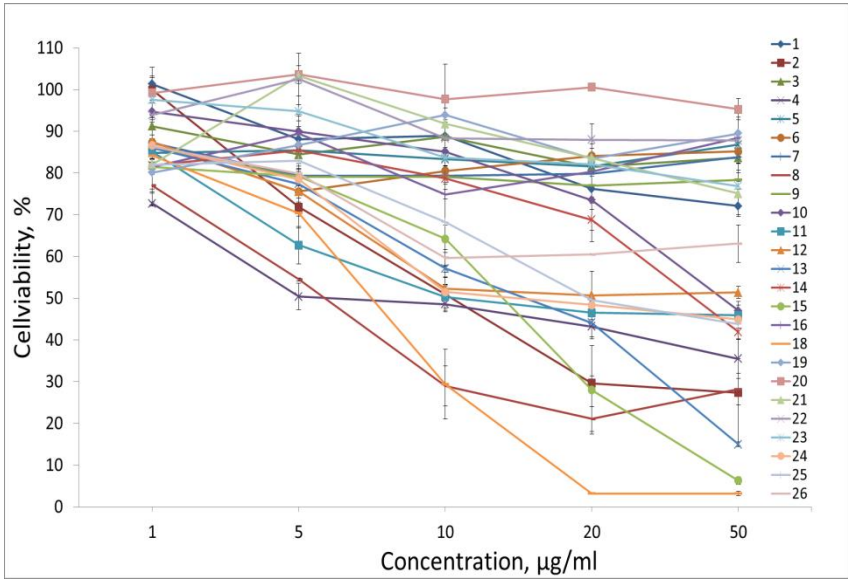


Antiproliferative activity of spiro-fused [3-azabicyclo[3.1.0]hexane]oxindoles against **CT26** cell lines for 24 (A) and 72 (B) h.

Results and discussion

Antiproliferative effect of synthesized compounds against cancer cell lines

Compound	IC_{50}, μg/mL									
	K562		HeLa		Jurkat		MCF-7		CT26	
	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
2	14±2	6±1	>40	5±3	8±2	3±1	>40	>40	>40	11±2
4	16±2	4±1	>40	4±3	4±1	3±1	>40	34±4	>40	6±2
8	15±1	4±1	>40	4±2	4±1	3±1	>40	>40	>40	6±1
15	16±2	10±1	15±1	8±1	7±2	8±2	>40	24±3	20±2	14±2
18	9±2	6±2	14±1	7±3	4±1	2±1	21±4	10±1	15±2	7±2
24	11±2	7±1	>40	8±1	9±2	4±1	>40	>40	>40	15±3
26	16±2	8±1	>40	8±1	9±2	3±1	>40	>40	>40	>40

IC_{50} values of most active spiro-fused [3-azabicyclo[3.1.0]hexane]oxindoles against **K562**, **HeLa**, **Jurkat**, **MCF-7** and **CT26** cell lines for 24 and 72 h.

Results and discussion

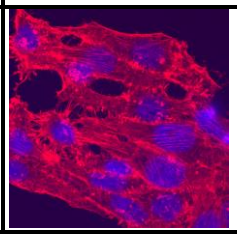
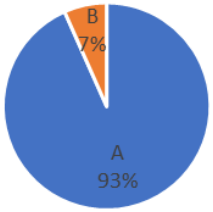
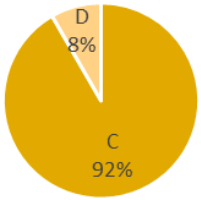
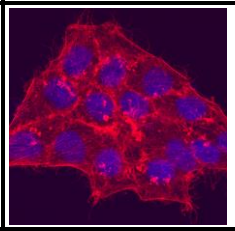
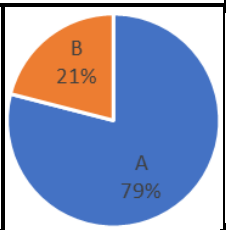
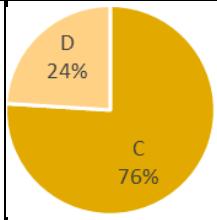
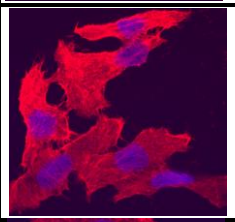
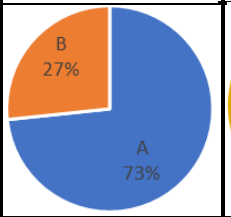
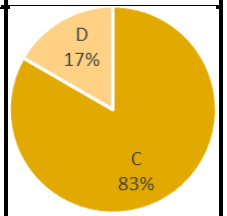
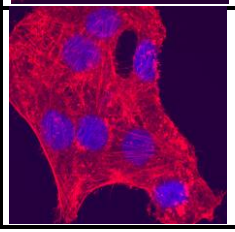
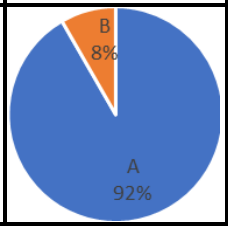
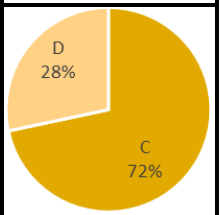
Actine cytoskeleton changes

The structure of the actin cytoskeleton of HeLa 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 by analysis of the experimental data that treatment with studing spiro-fused [3-azabicyclo[3.1.0]hexane]oxindoles led to significant changes of the actin cytoskeleton structure of tumor cells leading to the disappearance of stress fibers (granular actin was distributed diffusely in the cytoplasm in up to 38% of treated cells) and changes in the number of filopodia-like deformations (reduced from 93 % in control cells up to 64% after treatment).

Results and discussion

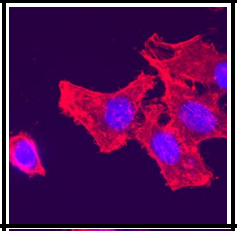
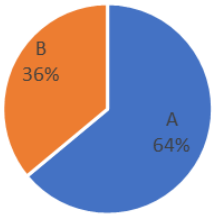
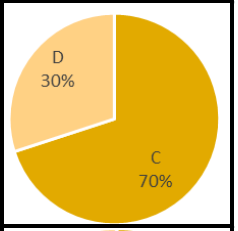
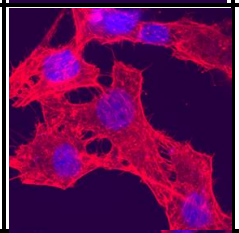
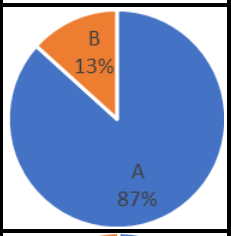
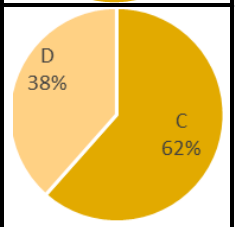
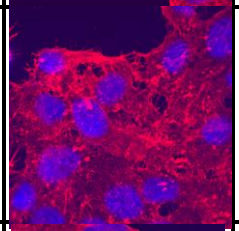
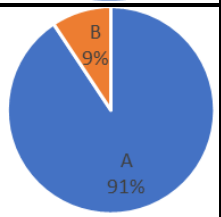
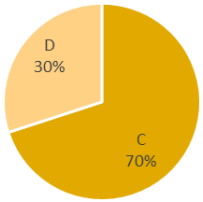
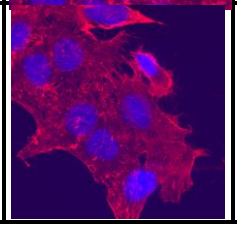
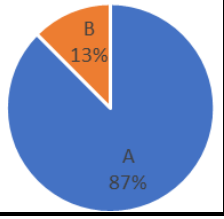
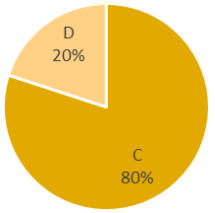
Actine cytoskeleton changes

Sample	I	II	III
HeLa control			
HeLa 2			
HeLa 4			
HeLa 8			

State of actin cytoskeleton of **HeLa** cells after treatment with compounds **2**, **4**, **8**, **15**, **18**, **24**, **26**. I: Images demonstrate the different stages of cell actin cytoskeleton. II: Pie charts demonstrate percentage of cells with filopodia-like deformations (A), and without filopodia-like deformations (B). III: Pie charts demonstrate percentage of cells with normal stress fibers (C) and disassembled stress fibers (D).

Results and discussion

Actine cytoskeleton changes

Sample	I	II	III
HeLa 15			
HeLa 18			
HeLa 24			
HeLa 26			

State of actin cytoskeleton of **HeLa** cells after treatment with compounds **2**, **4**, **8**, **15**, **18**, **24**, **26**. I: Images demonstrate the different stages of cell actin cytoskeleton. II: Pie charts demonstrate percentage of cells with filopodia-like deformations (A), and without filopodia-like deformations (B). III: Pie charts demonstrate percentage of cells with normal stress fibers (C) and disassembled stress fibers (D).

Results and discussion

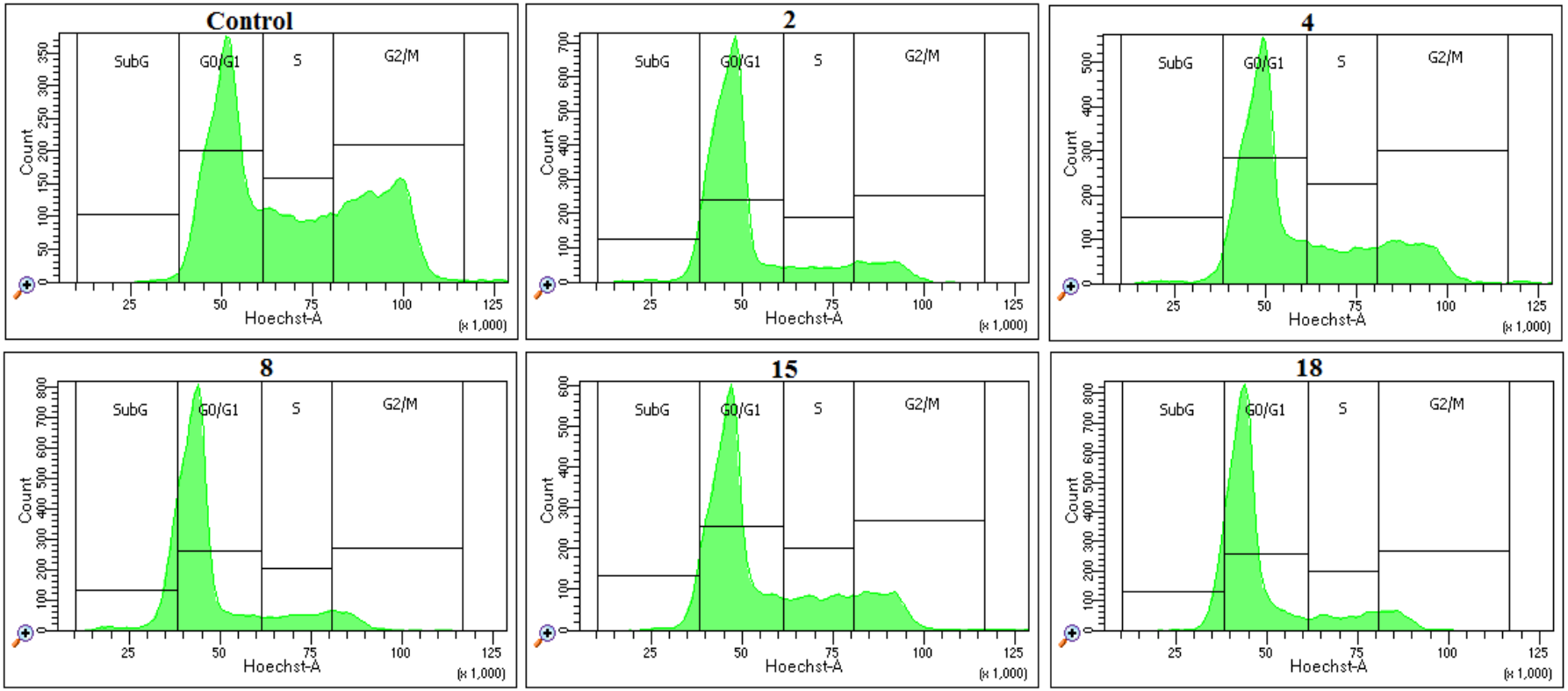
Cell cycle analysis

Analysis of the experimental results showed that screened compounds stop the K562 cell cycle in SubG1 and G0/G1 phases. So, after the impact of tested compounds at concentration 10 μg/mL for 24h, the percentage of cells in G0/G1 phase of cycle increased from 45.0% (control sample) up to 73.2% for compound **2**. The percentage of cells in SubG1 phase of cycle increased from 0.9% (control sample) up to 16.0% for compound **8**. Total percentage of cells in SubG1 and G0/G1 phases increased from 45.9% up to 78.7-81.3% for compounds **2**, **8** and **18**. The percentage of cells in the synthetic phase (S) of the cycle is also lower for treated (up to 9.5-15.9%) then untreated cells (19.4%). The percentage of cells in G2/M phase of the cycle decreased from 33.3% for untreated cells up to 7.2 and 7.9% for treated with compounds **8** and **18** respectively.

These findings indicate that tested compounds prevent cancer cells from starting DNA division.

Results and discussion

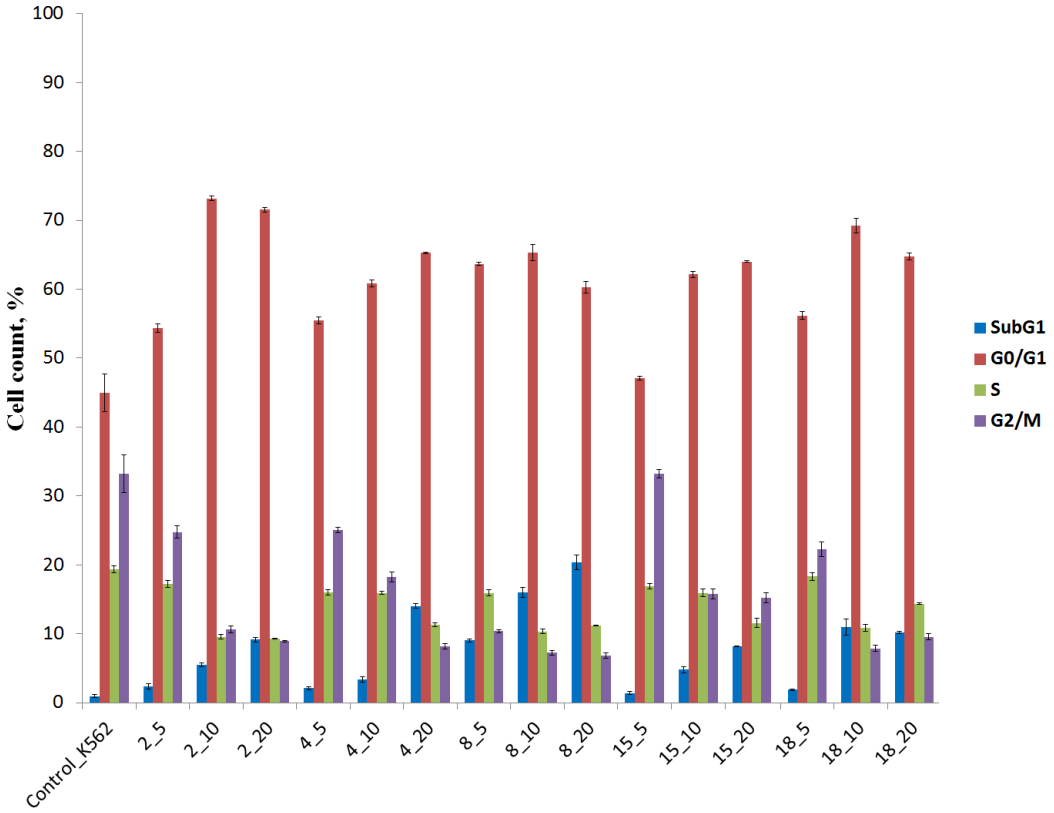
Cell cycle analysis



Effect of cycloadducts **2**, **4**, **8**, **15**, **18** at concentration 10 μg/mL on the distribution of **K562** cells in the cell cycle

Results and discussion

Cell cycle analysis



Effect of cycloadducts **2**, **4**, **8**, **15**, **18** at concentrations 5, 10, 20 μg/mL on the distribution of **K562** cells in the cell cycle

Results and discussion

Cell cycle analysis

Sample	SubG1	G0/G1	S	G2/M
Control_K562	0.9±0.2	45.0±2.8	19.4±0.5	33.3±2.7
2 (5 μg/mL)	2.3±0.4	54.4±0.6	17.2±0.5	24.8±0.9
2 (10 μg/mL)	5.5±0.3	73.2±0.3	9.5±0.3	10.6±0.5
2 (20 μg/mL)	9.1±0.3	71.6±0.3	9.3±0.1	8.9±0.1
4 (5 μg/mL)	2.1±0.2	55.5±0.5	16.0±0.3	25.1±0.4
4 (10 μg/mL)	3.4±0.3	60.9±0.5	15.9±0.2	18.2±0.7
4 (20 μg/mL)	14.0±0.3	65.3±0.1	11.3±0.3	8.2±0.4

Sample	SubG1	G0/G1	S	G2/M
8 (5 μg/mL)	9.0±0.2	63.7±0.2	15.9±0.5	10.4±0.3
8 (10 μg/mL)	16.0±0.7	65.3±1.2	10.3±0.4	7.2±0.3
8 (20 μg/mL)	20.3±1.1	60.3±0.9	11.2±0.1	6.8±0.3
15 (5 μg/mL)	1.4±0.2	47.1±0.3	16.9±0.4	33.2±0.6
15 (10 μg/mL)	4.8±0.5	62.2±0.5	15.9±0.6	15.8±0.7
15 (20 μg/mL)	8.1±0.1	64.0±0.1	11.6±0.7	15.2±0.7
18 (5 μg/mL)	1.9±0.1	56.2±0.6	18.3±0.5	22.2±1.1
18 (10 μg/mL)	11.0±1.2	69.3±1.1	10.8±0.5	7.9±0.4
18 (20 μg/mL)	10.1±0.2	64.7±0.5	14.4±0.1	9.6±0.5

Effect of cycloadducts **2**, **4**, **8**, **15**, **18** at concentration 5, 10 and 20 μg/mL on the distribution of **K562** cells in the cell cycle

Conclusions

A series of heterocyclic compounds containing 3-spiro[3-azabicyclo[3.1.0]hexane]oxindole framework have been studied for their antiproliferative activity against human K562, Jurkat, HeLa, MCF-7 as well as mouse CT26 cell lines. It was found that Jurkat cell line was the most sensitive to the screened compounds among the tested cell lines with IC_{50} ranging from 2±1 to 8±2 μg/mL (72 h). It was noticed that products with unsubstituted at nitrogen atom of oxindol unit were more active against all studied cell lines. At the same time among tested products bearing three phenyl substituents at cyclopropane unit were usually more effective. All the active compounds significantly reduce proliferative activity at concentrations less that 10 μg/mL. It was found that tested compounds have achieved significant cell-cycle perturbation with higher accumulation of cells in SubG1 and G0/G1 phases up to 78.7-81.3% (as compared to 45.9% in control sample). It was also found that actin filaments disappeared and granular actin was distributed diffusely in the cytoplasm in up to 38% of treated cells. Also we noticed that the number of cells with filopodium-like membrane protrusions was significantly reduced after treatment with some of tested compounds (from 93 % in control cells up to 64% after treatment), which indirectly suggests a decrease in cell motility. The obtained results support the antitumor effect of the studied compounds and encourage the extension of the study in order to improve the anticancer activity and reduce the toxicological risks of the obtained compounds.

Acknowledgments

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