The 9th International Electronic Conference on Medicinal Chemistry (ECMC 2023)
01–30 November 2023 | Online

Study of cytotoxicity of cyclopropa[a]pyrrolizidine and 3-azabicyclo[3.1.0]hexane derivatives spiro-fused with acenaphthylen-1(2H)-one and aceanthrylen-1(2H)-one fragments against tumor cell lines

Chaired by Dr. Alfredo Berzal-Herranz and Prof. Dr. Maria Emília Sousa

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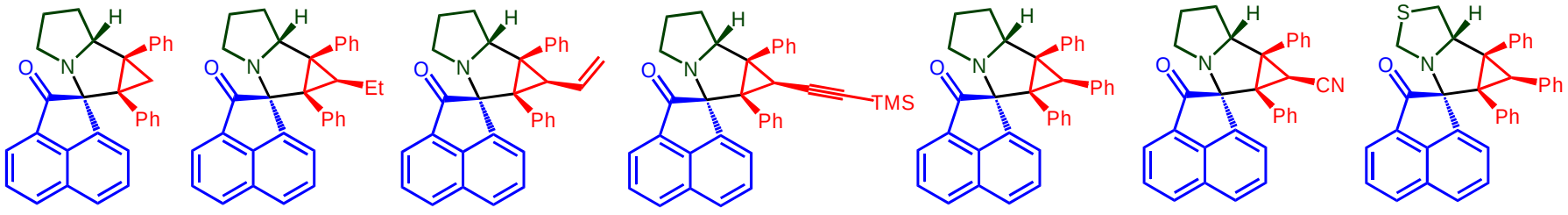
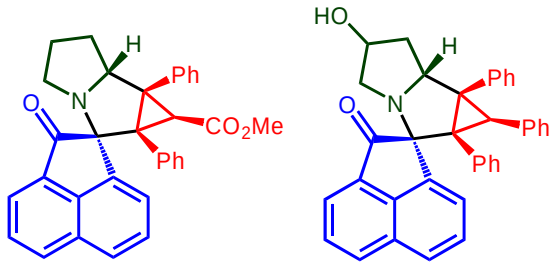


IC_{50} up to 5 μg/mL



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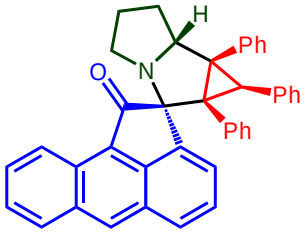

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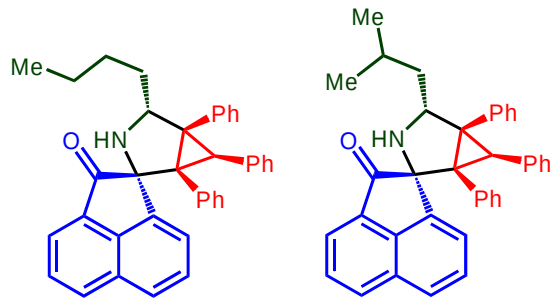


IC_{50} up to 9 μg/mL

Abstract: Oncological diseases are one of the most common public health problems and second leading cause of death after cardiovascular disease. Natural products or synthetic compounds inspired from natural products continue to be excellent sources for new drug candidates. Pyrrolizidines and 3-azabicyclo[3.1.0]hexanes are important classes of heterocyclic compounds with diverse pharmacological activities including anticancer activity. Here we report study of cytotoxicity of newly developed cyclopropa[a]pyrrolizidine and 3-aza-bicyclo[3.1.0]hexane derivatives spiro-fused with acenaphthylen-1(2H)-one and aceanthrylen-1(2H)-one fragments against tumor cell lines. The results of antiproliferative activity study showed that spiroadducts with either acenaphthylen-1(2H)-one or aceanthrylen-1(2H)-one framework have shown significant activity with IC_{50} up to 1 μg/mL. Confocal microscopy study showed diffuse distribution of granular actin in the cell cytoplasm with disappearance of actin filaments. Number of cells with filopodium-like membrane protrusions was also reduced after treatment with some of tested compounds. The obtained results support the antitumor effect of the studied compounds.

Keywords: cyclopropa[a]pyrrolizidine; 3-azabicyclo[3.1.0]hexane; acenaphthylen-1(2H)-one; aceanthrylen-1(2H)-one; in vitro antitumor activity; actin cytoskeleton structure

Introduction

Cancer is one of the most frequent health problems worldwide and follows after cardiovascular diseases the second leading cause of mortality. The development of cytostatic agents still remains an essential task for cancer therapy despite the growing application of targeted drugs and immunotherapy methods. Development of drug resistance requires the generation of new chemical entities that are not just "classical" drugs derivatives, but arising from compounds of new nature. Natural products or artificial compounds created on the basis of natural products are still excellent sources of new drug candidates [1]. Today many of the most successful applicable anticancer drugs either are natural origin compounds, or created on the basis of thereof [2].

3-Azabicyclo[3.1.0]hexanes and pyrrolizidines are important classes of heterocyclic compounds with diverse pharmacological and biological activities. For example, 3-azabicyclo[3.1.0]hexanes exhibit anti-neuroinflammatory **1** [3], anti-neurodegenerative **2** [4], and antiviral **3** [5] activity. Pyrrolizidine is the main structural fragment of many organic compounds exhibiting various biological activities, in particular, anticoagulant **4** [6], anticancer **5** [7], and anti-HIV **6** [8] activity (Fig. 1).

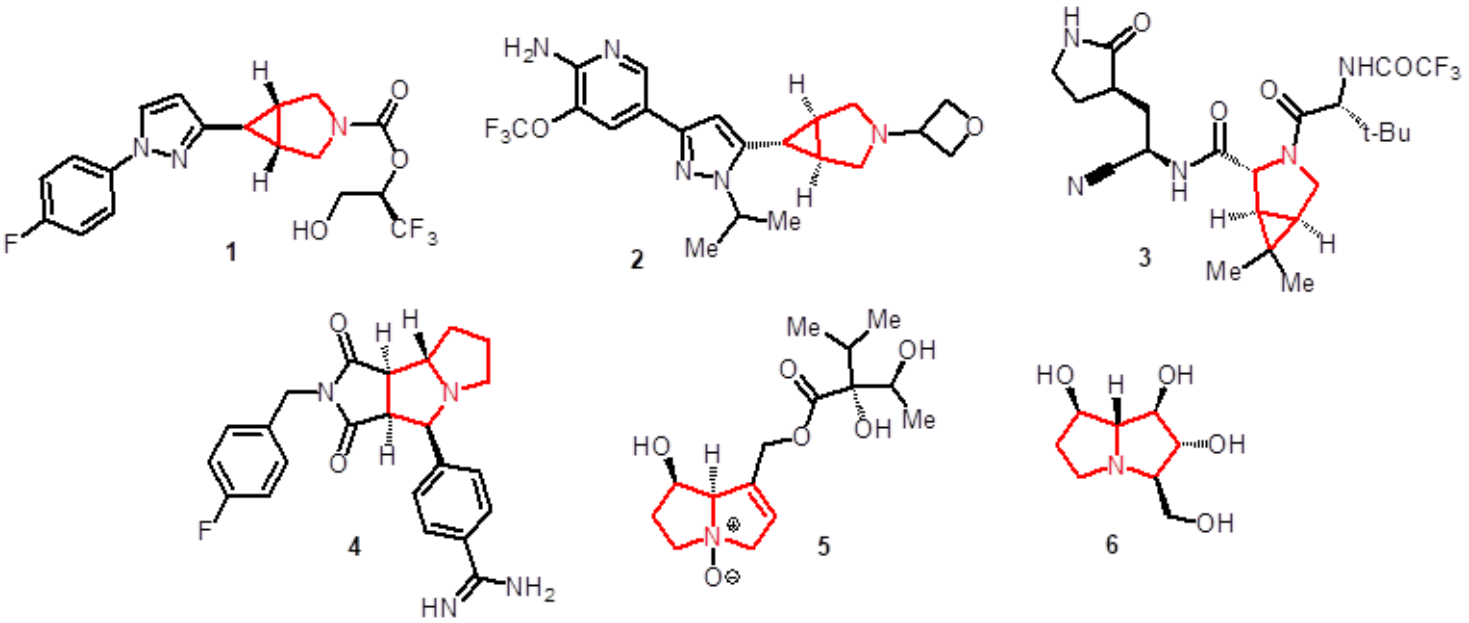
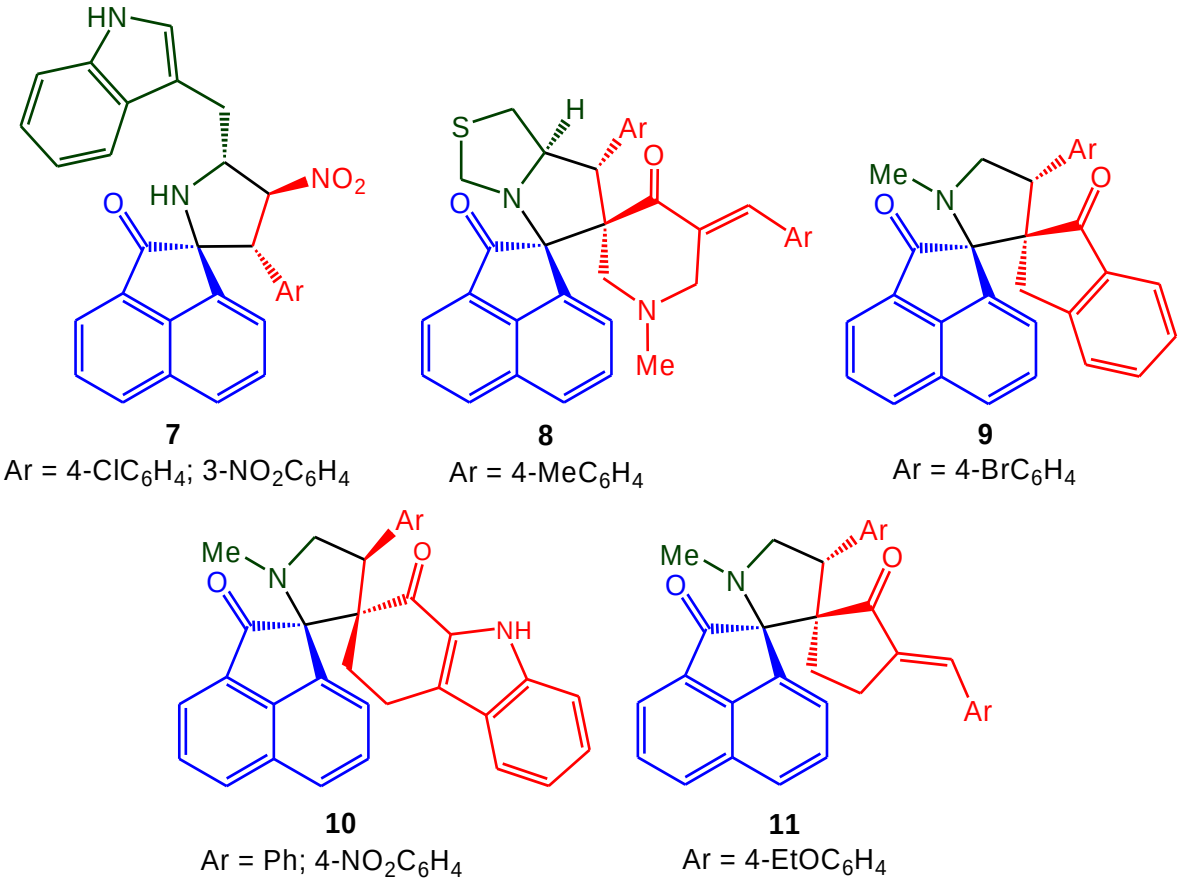


Figure 1. Selected examples of biologically active 3-azabicyclo[3.1.0]¬hexanes and pyrrolizidines.

Pyrrolidines spiro-fused to an acenaphthylen-1(2*H*)-one display a variety of pharmacological effects including cholinesterase inhibitory activity **7** and **8** [9, 10], antimycobacterial activity **9** [11], antimicrobial activity **10** [12], and may also be of interest in the treatment of osteoarthritis **11** [13] (Fig. 2). In addition, compounds with a spiro[acenaphthylene-1,2'-pyrrolidin]-2-one moiety have been described as having anticancer, α-amylase inhibitory, antitubercular and antifungal activity.



7
Ar = 4-ClC_6H_4; 3-$NO_2C_6H_4$

8
Ar = 4-MeC_6H_4

9
Ar = 4-BrC_6H_4

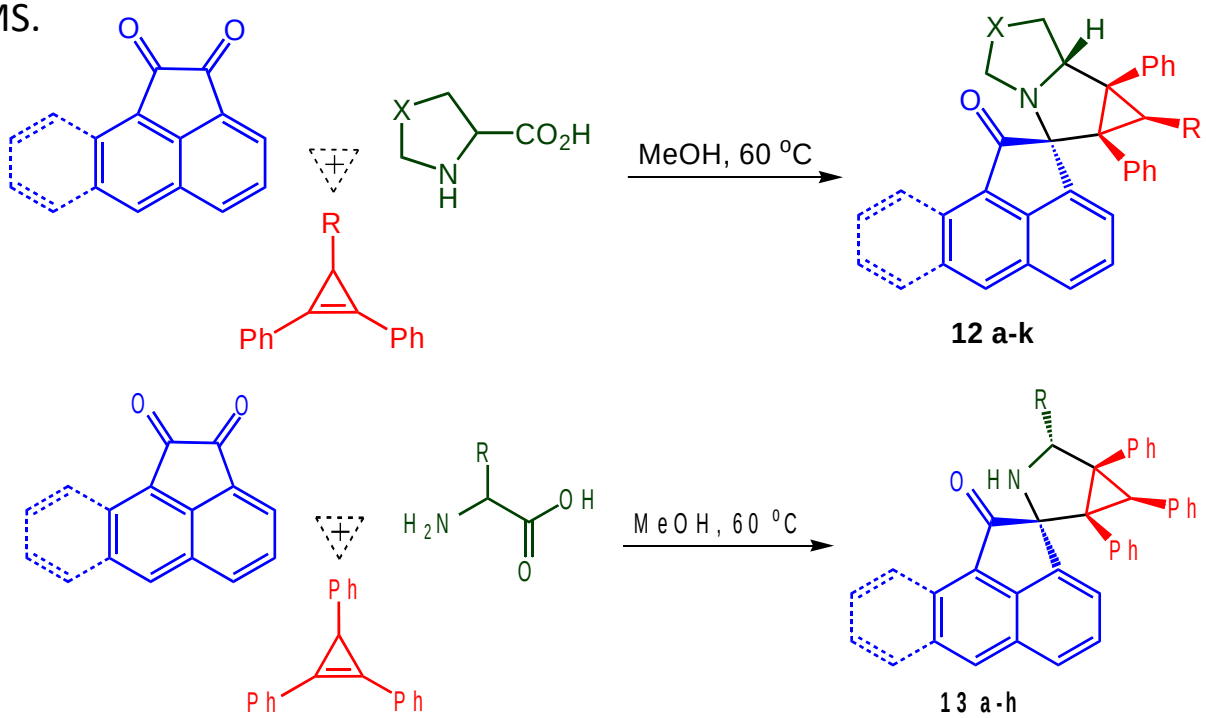
10
Ar = Ph; 4-$NO_2C_6H_4$

11
Ar = 4-$EtOC_6H_4$

Figure 2. Representative active 2H-spiro[acenaphthylene-1,2'-pyrrolidin]-2-ones **7**–**11**

Results and discussion

Synthesis of cyclopropa[*a*]pyrrolizidine (**12**) and 3-azabicyclo[3.1.0]hexane (**13**) derivatives spiro-fused with acenaphthylen-1(2*H*)-one and aceanthrylen-1(2*H*)-one fragments was carried out by previously developed by us methodology [14, 15] using one-pot three-component [3+2]-cycloaddition reaction of cyclopropenes, α-amino acids and acenaphthylene-1,2-dione (or aceanthrylene-1,2-dione) according to the scheme 1. All compounds were fully characterized by NMR, HRMS.



Scheme 1. Synthesis of racemic spiro-adducts **12a–k** and **13a–h**

Results and discussion

Antiproliferative activity of studied spiro-adducts with cyclopropa[*a*]pyrrolizidine (**12**) and 3-azabicyclo[3.1.0]hexane (**13**) fragments against human cervical carcinoma (HeLa), human erythroleukemia (K562), human (Skmel) and murine (B16) melanoma cell lines was evaluated *in vitro* using the standard MTS assay for 24 and 72 h. Structures of tested compounds are presented at Figures 3-4, while the results of these investigations are presented in Figures 5-8.

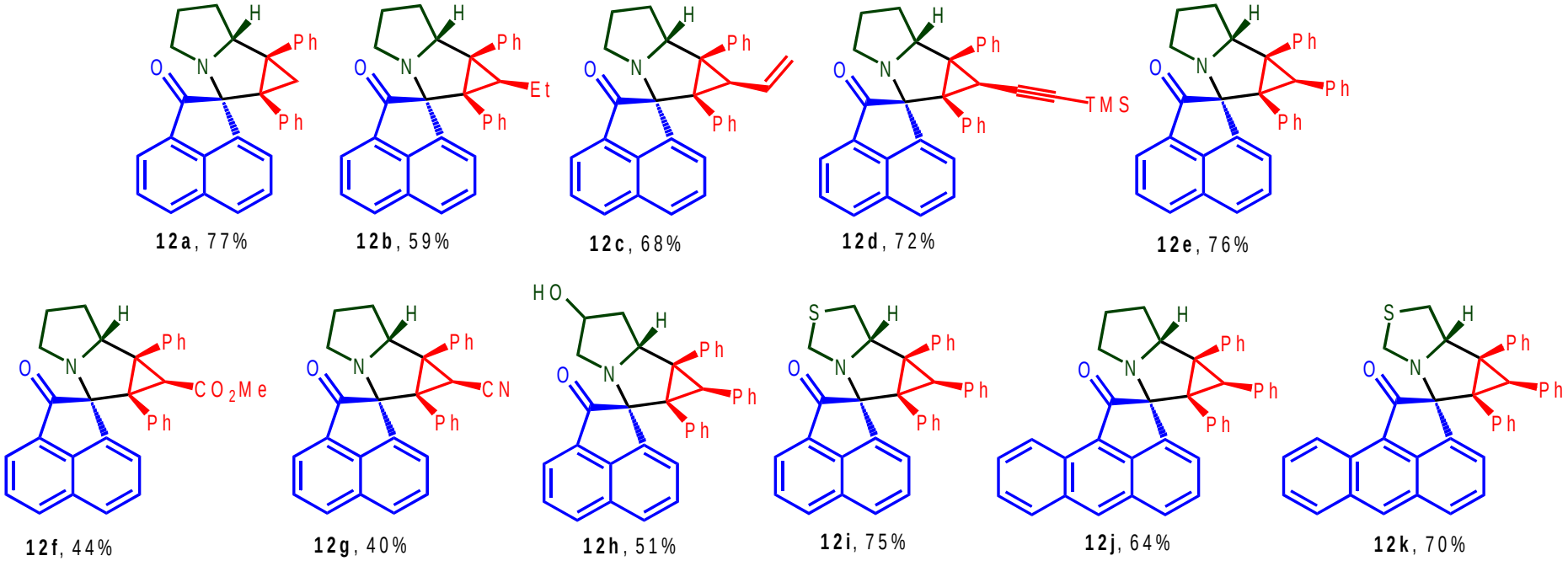


Figure 3. Structures of tested spiro-fused cyclopropa[*a*]pyrrolizidine adducts **12a–k**

Results and discussion

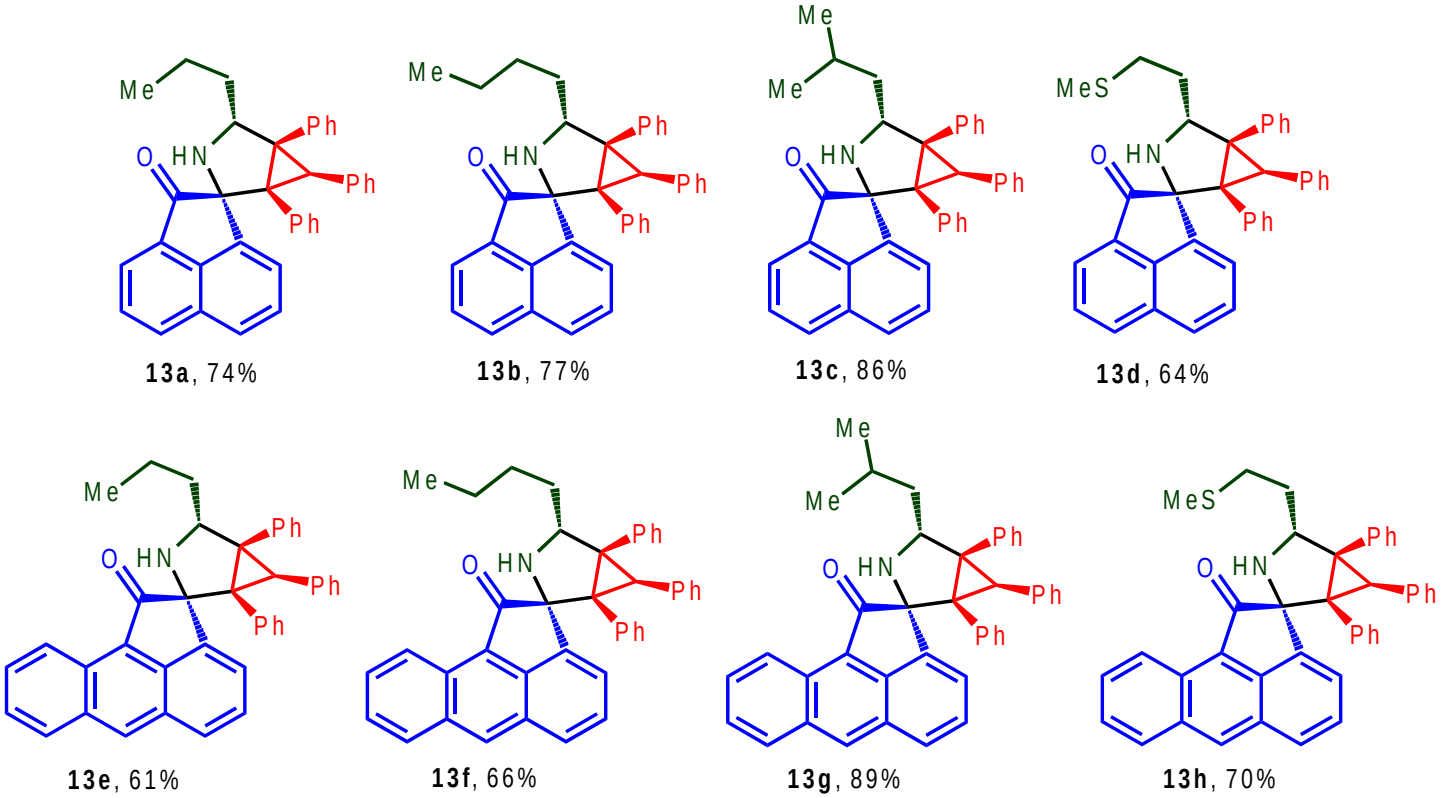


Figure 4. Structures of tested spiro-fused 3-azabicyclo[3.1.0]hexane adducts **13a–h**

Results and discussion

Studying of antiproliferative activity indicate that both spiroadducts **12** and **13** were more active against human erythroleukemia (K562) and murine melanoma (B16) cell lines with the most potent products showing IC_{50} ranging from around 10 µg/mL for **13b**, **13c** to less then 1 µg/mL for **12f**, **12h** after treatment for 72 h.

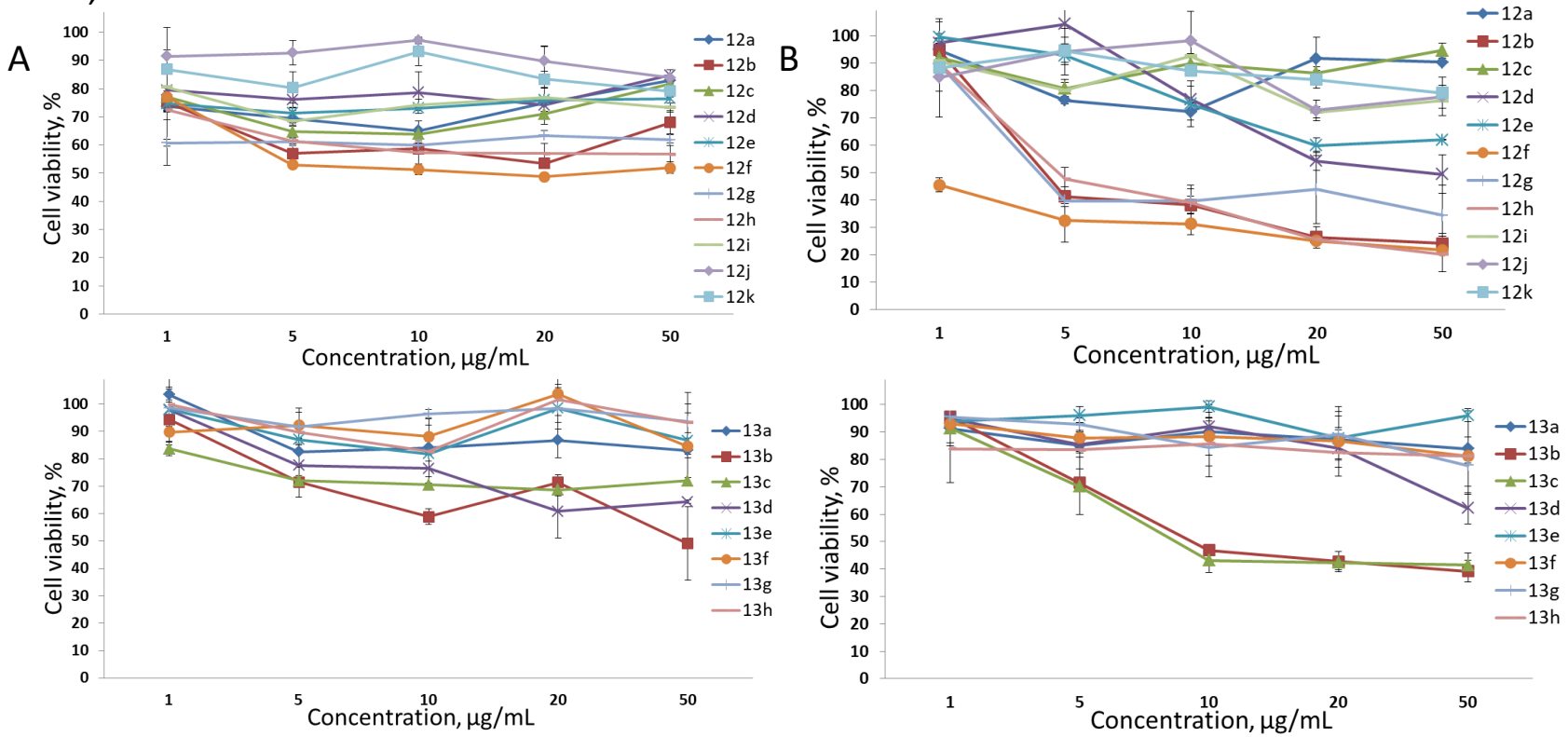


Figure 5. Antiproliferative activity of studied compounds against K562 cell line after 24 (A) and 72h (B)

Results and discussion

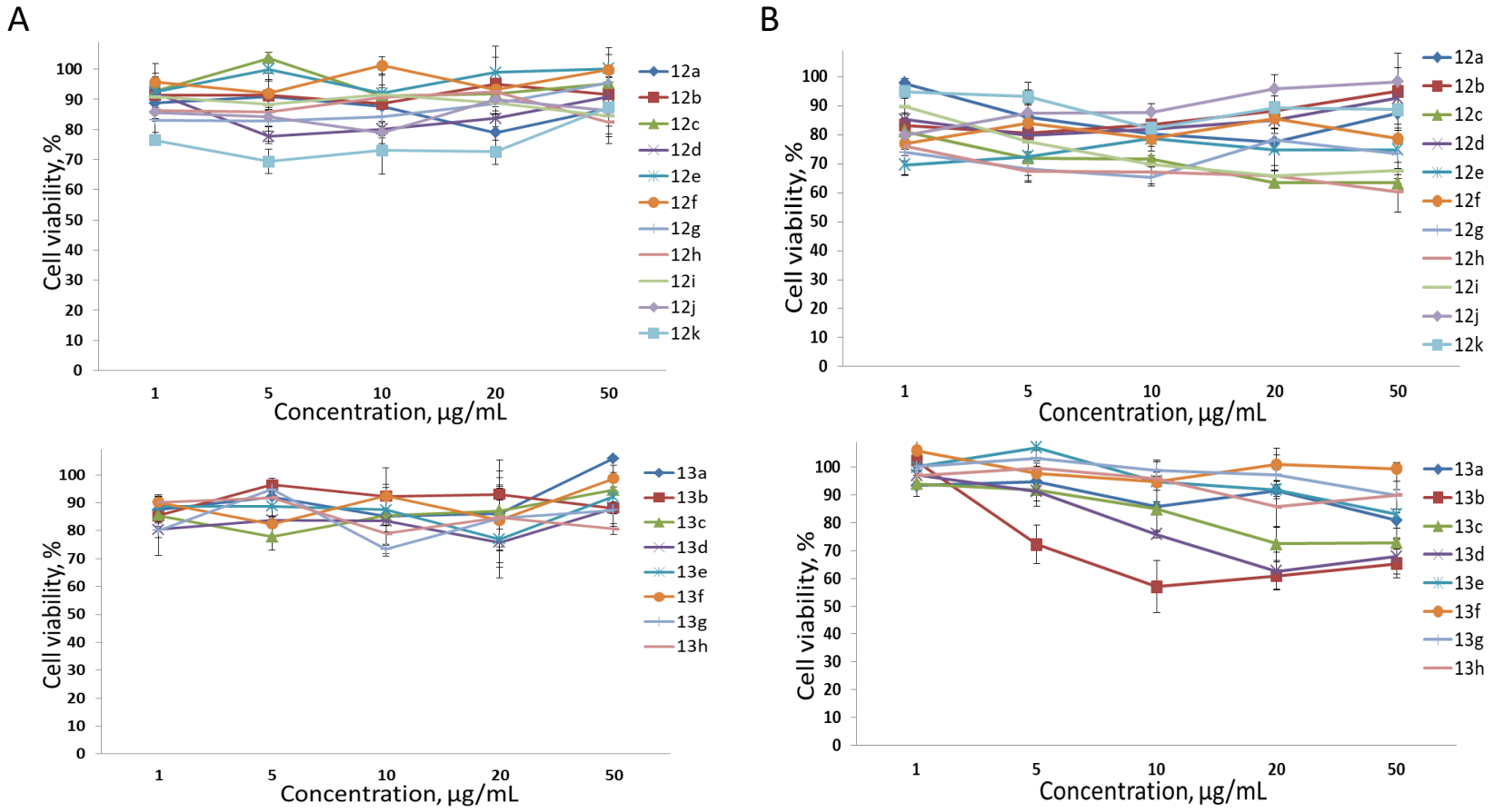


Figure 6. Antiproliferative activity of studied compounds against HeLa cell line after 24 (A) and 72h (B)

Results and discussion

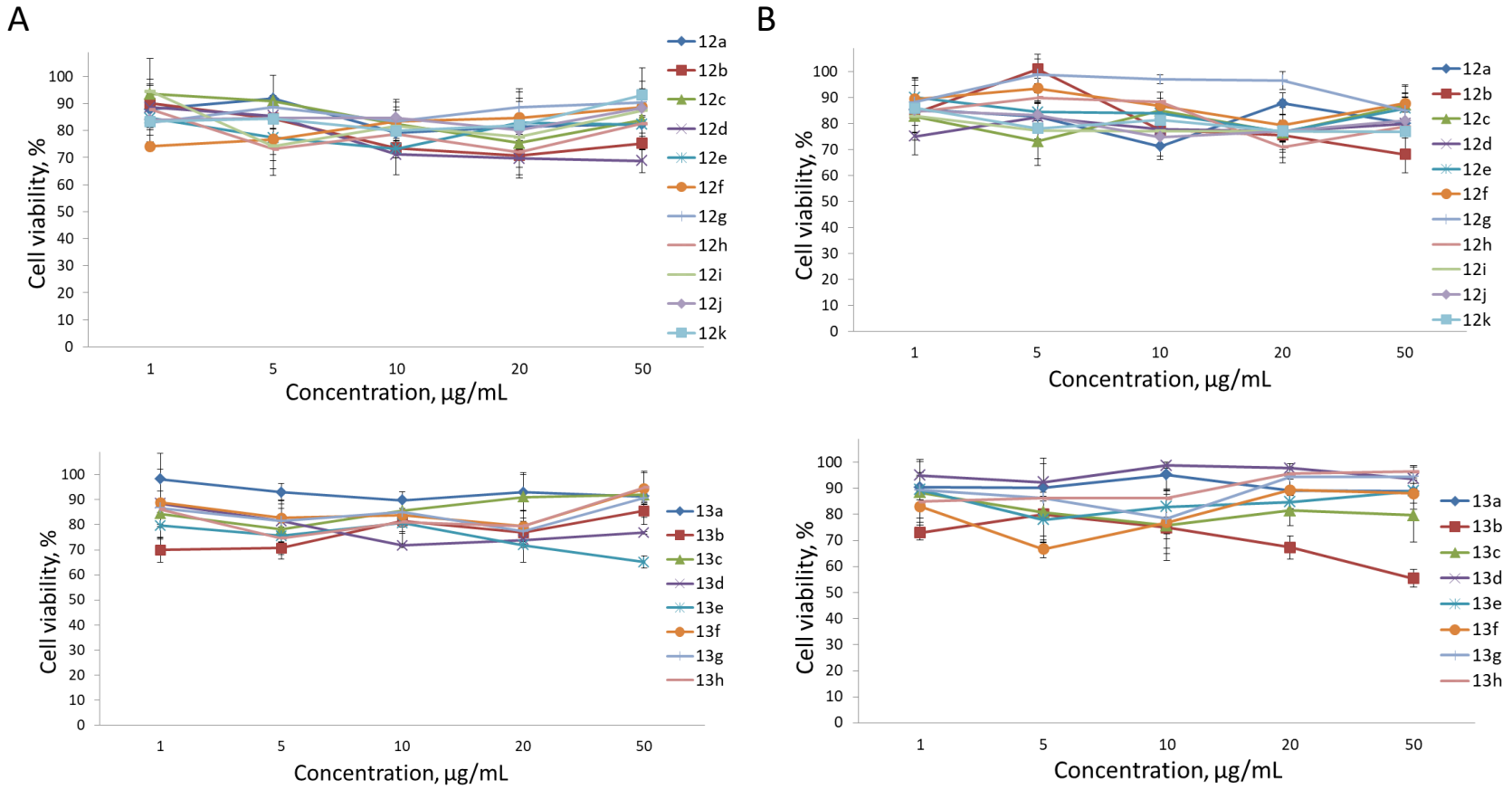


Figure 7. Antiproliferative activity of studied compounds against Sk-mel-2 cell line after 24 (A) and 72h (B)

Results and discussion

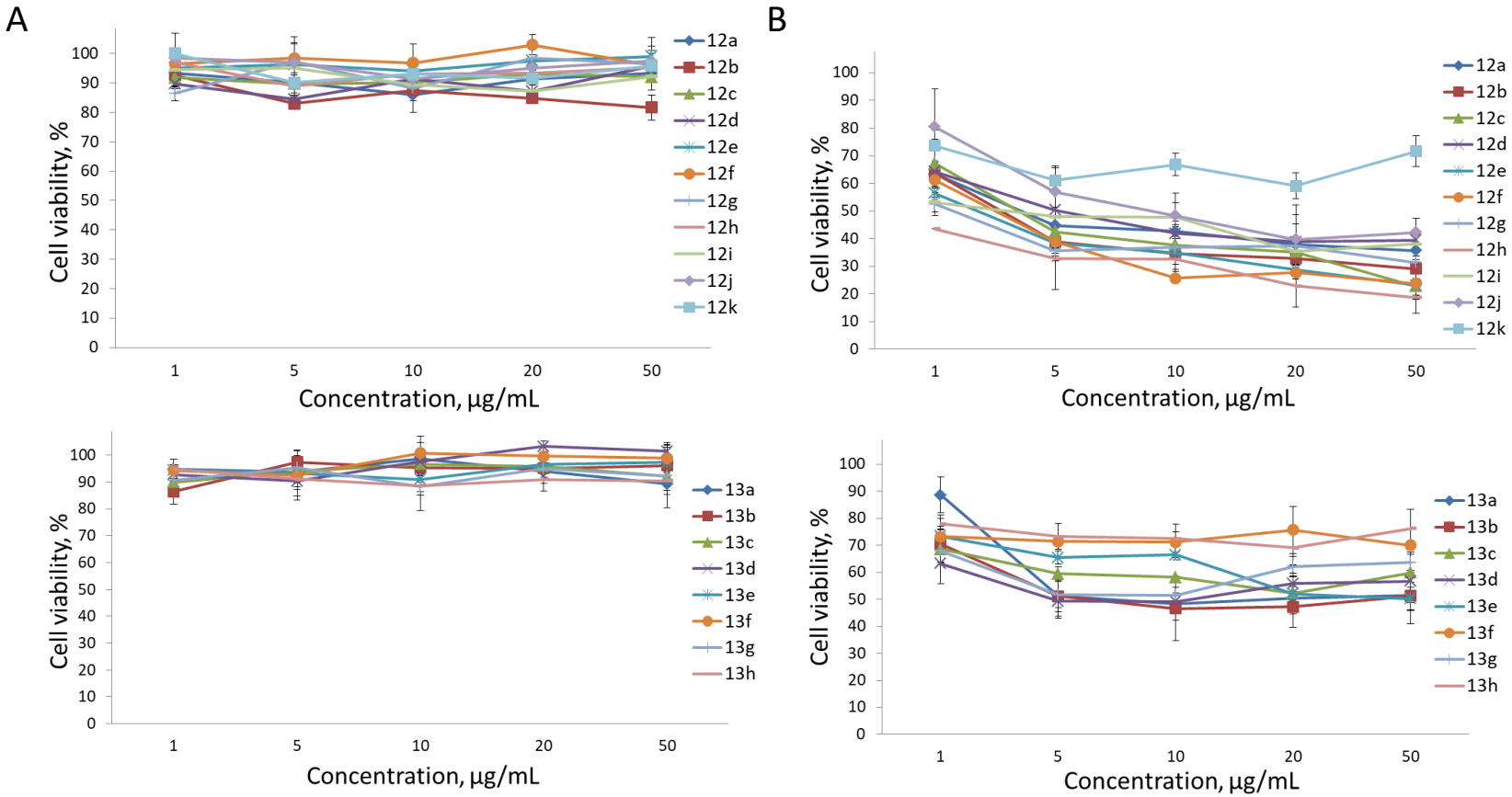


Figure 8. Antiproliferative activity of studied compounds against B16 cell line after 24 (A) and 72h (B)

Results and discussion

As could be seen from the data above generally spiro-adducts with cyclopropa[*a*]pyrrolizidine (**12**) moiety affect at cell lines strongly compared to that with 3-azabicyclo[3.1.0]hexane (**13**) fragments. Spiro-fused with acenaphthylen-1(2H)-one products are usually more active than those with aceanthrylen-1(2H)-one fragments.

Indeed cyclopropa[*a*]pyrrolizidines with hydroxy-substituted pyrrolidine ring (**12h**) or ethyl- (**12b**), nitril- (**12g**) and carboxymethyl- (**12f**) substituted at cyclopropane ring demonstrated significant activity with IC_{50} 5 ± 1 (**12b**,**g**,**h**) and less then 1 μg/mL (**12f**) after treatment K562 cells for 72 h, while all the products (except for **12k**) demonstrated significant activity with IC_{50} ranging from 5 (**12a**–**i**) to less then 1 μg/mL (**12h**) after treatment B16 cells for 72 h.

Among the tested 3-azabicyclo[3.1.0]hexanes only buthyl- (**13b**) and isobuthyl- (**13c**) substituted products have shown significant activity (IC_{50} 9 ± 1 μg/mL after 72 h treatment).

Representative compounds **12b,f,g,h** and **13b,c** showed a comparable or stronger inhibitory effect (IC_{50} = 1 to 10 μg/mL, that equal 2 to 17 μM) against human leukemia cells K562 compared with cisplatin (IC_{50} = 25-45 μM) [16,17].

Results and discussion

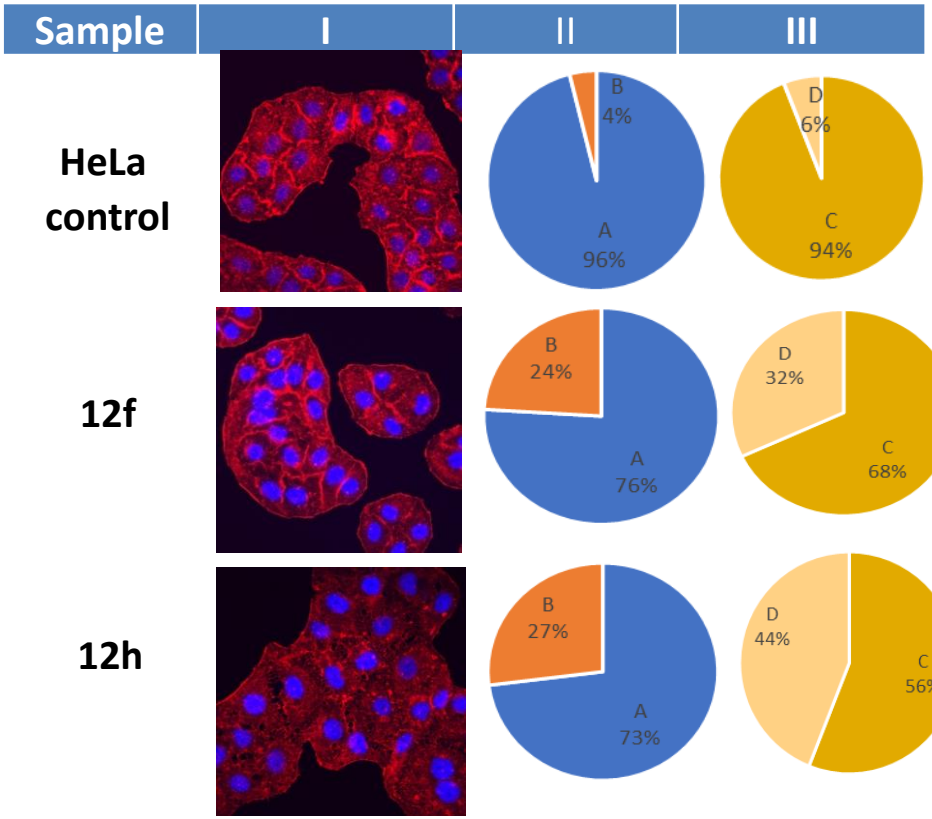
Actin cytoskeleton plays an essential role in vital cellular processes such as cell adhesion, migration, morphogenesis and may be used as an additional target for chemotherapeutic intervention [16, 18]. HeLa cell line is widely used to the actin cytoskeleton structure study. This cell line is characterized by the presence of actin stress fibers and filopodia [19].

Based on obtained data compounds **12f, 12h**, **13b**, **13c** and **13d** were selected for further evaluation of the effects on cytoskeletal morphology of HeLa cells. Actin cytoskeleton structure was analyzed after the impact of chosen compounds by the presence of filopodia-like protrusions and the availability of stress fibers (Figure 9).

It was found that treatment with these compounds have led to substantial alteration in the tumor cells actin cytoskeleton structure that lead to the changes in the number of filopodia-like deformations (decreases from 96% in control to 69%) and stress fibers disappearance (decreases from 94% in control to 58%). This effect was determined for most antiproliferatively active at HeLa cell line compound **13b**.

It is interesting to note that even much less antiproliferatively active at HeLa cell line compounds **12f, 12h**, **13c** and **13d** have led to meaningful alterations in actin cytoskeleton structure (number of filopodia-like deformations decreases to 88-73% while number of stress fibers decreases to 82-56%.

Results and discussion



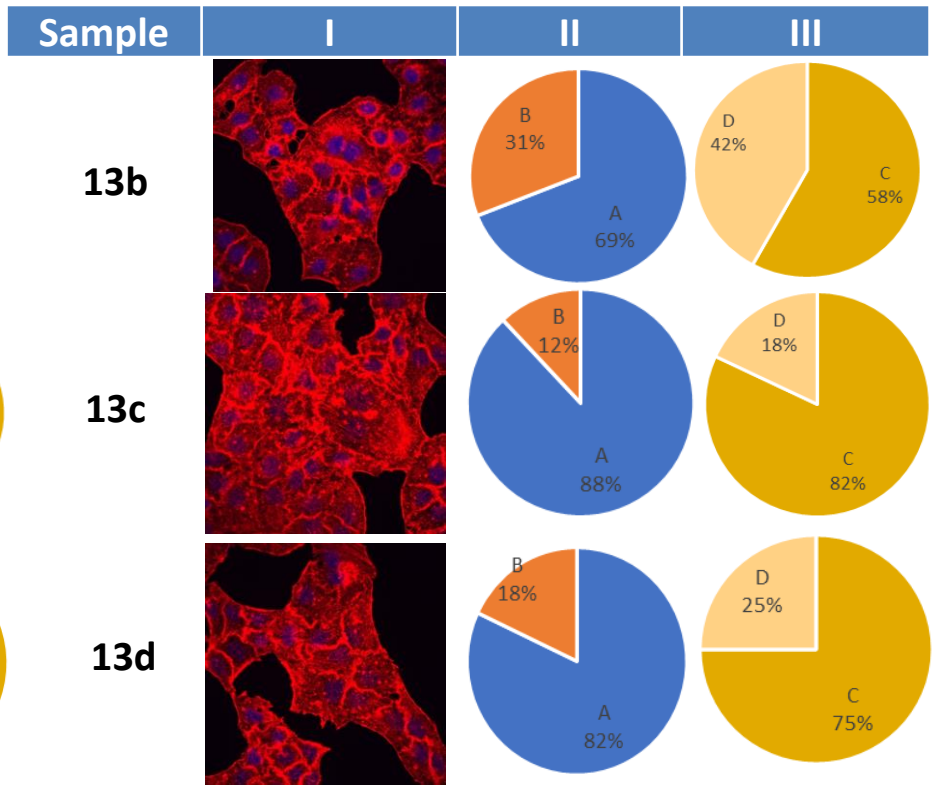


Figure 9. State of actin cytoskeleton of HeLa cells after treatment with pyrrolo[3,4-d]isoxazoles **12f, 12h**, **13b**, **13c** and **13d** . I: Images demonstrate the different stages of cell actin cytoskeleton. II: Pie charts demonstrate percentage of cells with filopodia-like deformations (A) and without filopodia-like deformations (B). III: Pie charts demonstrate percentage of cells with normal stress fibers (C) and disassembled stress fibers (D).

Conclusions

The results of antiproliferative activity study showed that cyclopropa[a]pyrrolizidines and 3-aza-bicyclo[3.1.0]hexanes spirosubstituted with acenaphthylen-1(2H)-one and aceanthrylen-1(2H)-one fragments have shown significant activity. Spiro-adducts with cyclopropa[*a*]pyrrolizidine (**12**) moiety are usually more active compared to that with 3-azabicyclo[3.1.0]hexane (**13**) fragments. Spiro-fused with acenaphthylen-1(2H)-one products are usually more active than those with aceanthrylen-1(2H)-one fragments.

Cyclopropa[*a*]pyrrolizidines with hydroxy-substituted pyrrolidine ring (**12h**) or ethyl- (**12b**), nitril- (**12g**) and carboxymethyl- (**12f**) substituted at cyclopropane ring demonstrated significant activity with IC_{50} 5 ± 1 (**12b**,**g**,**h**) and less then 1 μg/mL (**12f**) after treatment K562 cells for 72 h, while all the products (except for **12k**) demonstrated significant activity with IC_{50} ranging from 5 (**12a–i**) to less then 1 μg/mL (**12h**) after treatment B16 cells for 72 h. Among the tested 3-azabicyclo[3.1.0]hexanes only buthyl- (**13b**) and isobuthyl- (**13c**) substituted products have shown significant activity (IC_{50} 9 ± 1 μg/mL after 72 h treatment).

Confocal microscopy revealed that actin filaments disappeared and granular actin was distributed diffusely in the cytoplasm of up to 44% of HeLa cells after their treatment with tested compounds. The number of HeLa cells with filopodium-like membrane protrusions was reduced significantly after treatment (from 96% in control to 69% in treated cells). Notably even much less cytotoxic compounds still have meaningful effect on organization of cytoskeleton structure.

Acknowledgments

This work was supported by Ministry of Education and Science of the Russian Federation (projects FSRM-2023-0005 and FSEN-2023-0002) and Russian Science Foundation (RSF 20-15-00332).

References

1. Chupakhin, E., Babich, O., Prosekov, A., Asyakina, L., Krasavin, M. Molecules. **2019**, 24, 4165
2. Newman, D.J., Cragg, G.M. J. Nat. Prod. **2020**, 83, 770
3. McAllister, L.A., Butler, C.R., Mente, S., O'Neil, S.V., Fonseca, K.R., Piro, J.R., Cianfrogna, J.A., Foley, T.L., Gilbert, A.M., Harris, A.R., Helal, C.J., Johnson, D.S., Montgomery, J.I., Nason, D.M., Noell, S., Pandit, J., Rogers, B.N., Samad, T.A., Shaffer, C.L., da Silva, R.G., Uccello, D.P., Webb, D., Brodney, M.A. J. Med. Chem. **2018**, 61, 3008.
4. Patel, S., Meilandt, W.J., Erickson, R.I., Chen, J., Deshmukh, G., Estrada, A.A., Fuji, R.N., Gibbons, P., Gustafson, A., Harris, S.F., Imperio, J., Liu, W., Liu, X., Liu, Y., Lyssikatos, J.P., Ma, C., Yin, J., Lewcock, J.W., Siu, M. J. Med. Chem. **2017**, 60, 8083.
5. Luttens, A., Gullberg, H., Abdurakhmanov, E., Vo, D.D., Akaberi, D., Talibov, V.O., Nekhotiaeva, N., Vangeel, L., De Jonghe, S., Jochmans, D., Krambrich, J., Tas, A., Lundgren, B., Gravenfors, Y., Craig, A.J., Atilaw, Y., Sandstrom, A., Moodie, L.W.K., Lundkvist, A., van Hemert, M.J., Neyts, J., Lennerstrand, J., Kihlberg, J., Sandberg, K., Danielson, U.H., Carlsson, J. J. Am. Chem. Soc. **2022**, 144, 2905.
6. Schweizer, E., Hoffmann-Röder, A., Olsen, J.A., Seiler, P., Obst-Sander, U., Wagner, B., Kansy, M., Banner, D.W., Diederich, F. Org. Biomol. Chem. **2006**, 4, 2364.
7. Bhutani, P., Joshi, G., Raja, N., Bachhav, N., Rajanna, P.K., Bhutani, H., Paul, A.T., Kumar, R.U.S. J. Med. Chem. **2021**, 64, 2339.
8. Moreira, R., Pereira, D.M., Valentao, P., Andrade, P.B. Int. J. Mol. Sci. **2018**, 19, 1668.
9. Arumugama, N., Almansoura, A.I., Kumara, R.S., Altafa, M., Padmanabanc, R., Sureshbabuc, P., Angamuthuc, G., Kotreshad, D., Manohare, T.S., Venketeshe, S. Bioorg. Chem. **2018**, 79, 64.
10. Sivakumar, S., Kumar, R.R., Ashraf, Ali M., Choon, T.S. Eur. J. Med. Chem. **2013**, 65, 240.
11. Wei, A.C., Ashraf, Ali M., Yoon, Y.K., Ismail, R., Choon, T.S., Kumar, R.S. Bioorg. Med. Chem. Lett. **2013**, 23, 1383.
12. Periyasami, G., Raghunathan, R., Surendiran, G., Mathivanan, N. Eur. J. Med. Chem. **2009**, 44, 959.
13. Xu, W., Xie, Y., Wang, Q., Wang, X., Luo, F., Zhou, S., Wang, Z., Huang, J., Tan, Q., Jin, M., Qi, H., Tang, J., Chen, L., Du, X., Zhao, C., Liang, G., Chen, L. Sci. Rep. **2016**, 6, 24042.
14. Filatov, A.S.; Knyazev, N.A.; Molchanov, A.P.; Panikorovsky, T.L.; Kostikov, R.R.; Larina, A.G.; Boitsov, V.M.; Stepakov, A.V. J. Org. Chem. **2017**, 82, 959–975.
15. Filatov, A.S., Selivanov, S.I., Shmakov, S.V., Larina, A.G., Boitsov, V.M., Stepakov, A.V. Synthesis **2022**, 54, 1803
16. Zhou, L.-M., Qu, R.-Y., Yang, G.-F. Expert Opinion on Drug Discovery. **2020**, 1–23.
17. Shmakov, S.V., Latypova, D.K., Shmakova, T.V., Rubinshtein, A.R., Chukin, M.V., Zhuravskii, S.G., Knyazev, N.A., Stepakov, A.V., Galagudza, M.G., Boitsov, V.M. Int. J. Mol. Sci. **2022**, 23, 10759
18. Tojkander, S.; Gateva, G.; Lappalainen, P. J. Cell. Sci. **2012**, 125, 1855–1864
19. Aseervatham, J. Biology **2020**, 9, 385