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Study of cytotoxicity of pyrrolo-[3,4-d]isoxazoles against tumor cell lines

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

pharmaceuticals MDPI

Vitali Boitsov [1,*], **Stanislav Shmakov** [1], **and Alexander Stepakov** [2]

[1] Saint Petersburg National Research Academic University of the Russian Academy of Sciences, Saint Petersburg, 194021, Russia;
[2] Saint Petersburg State University, Saint Petersburg, 199034, Russia.

* Corresponding author: bovitali@yandex.ru

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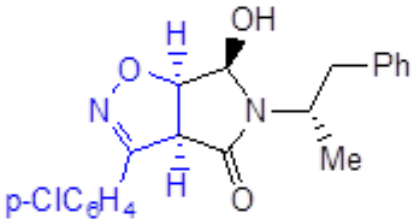
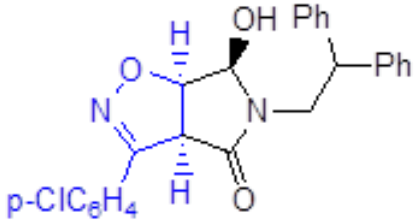
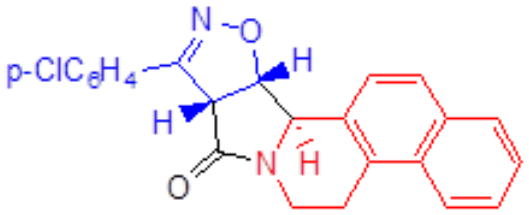


IC_{50} up to 14 μg/mL



IC_{50} up to 8 μg/mL



IC_{50} up to 7 μg/mL

Abstract: Cancer is a major public health problem and after cardiovascular disease it is second most common cause of death. Natural products and inspired by them synthetic products are excellent sources for new drug candidates. Oxazole and isoxazole structural fragments containing oxygen and nitrogen atoms are considered prime scaffolds for drug discovery. Such in field of medicinal chemistry these compounds could readily bind with a variety of enzymes and receptors in biological systems and show broad biological activities. Series of heterocyclic compounds containing pyrrolo[3,4-d]isoxazole framework was studied for their antiproliferative activity against human cervical carcinoma (HeLa), murine fibroblast (3T3) and SV-40 transformed murine fibroblast (3T3-SV40) cell lines. Confocal microscopy revealed that granular actin was distributed diffusely in the cytoplasm in up to 71% of treated cells after their treatment with tested compounds while actin filaments were disappeared. Number of cells with filopodium-like membrane protrusions was significantly reduced after treatment with some of tested compounds (from 92 % in control cells up to 18% after treatment). The obtained results support the antitumor effect of the studied compounds and encourage further study to improve the anticancer activity and reduce the toxicological risks of obtained compounds.

Keywords: pyrrolo[3,4-d]isoxazoles; in vitro antitumor activity; actin cytoskeleton structure

Introduction

Cancer is one of the most frequent health problems worldwide and after cardiovascular diseases it is the second leading cause of mortality. Despite the growing use of targeted drugs and methods of immunotherapy, the cytostatic agents development remains an essential task for therapy of cancer. The generation of new chemical entities is necessary to combat drug resistance. Natural products or artificial compounds created based on them are excellent sources of new drug candidates. Today many of the most successful applicable anticancer drugs either are natural origin compounds, or created on the basis of thereof.

Isoxazole and oxazole rings containing nitrogen and oxygen atoms are considered prime scaffolds for the drug discovery due to their structural features that allow for multiple weak non-covalent interactions such as hydrogen bonds, coordination bonds, π-π stacking, hydrophilic interactions and so on. Such in field of medicinal chemistry these compounds could readily bind with a variety of enzymes and receptors in biological systems and show broad biological activities like antibacterial, antifungal, antiviral, antitubercular, anticancer, anti-inflammatory and so on.

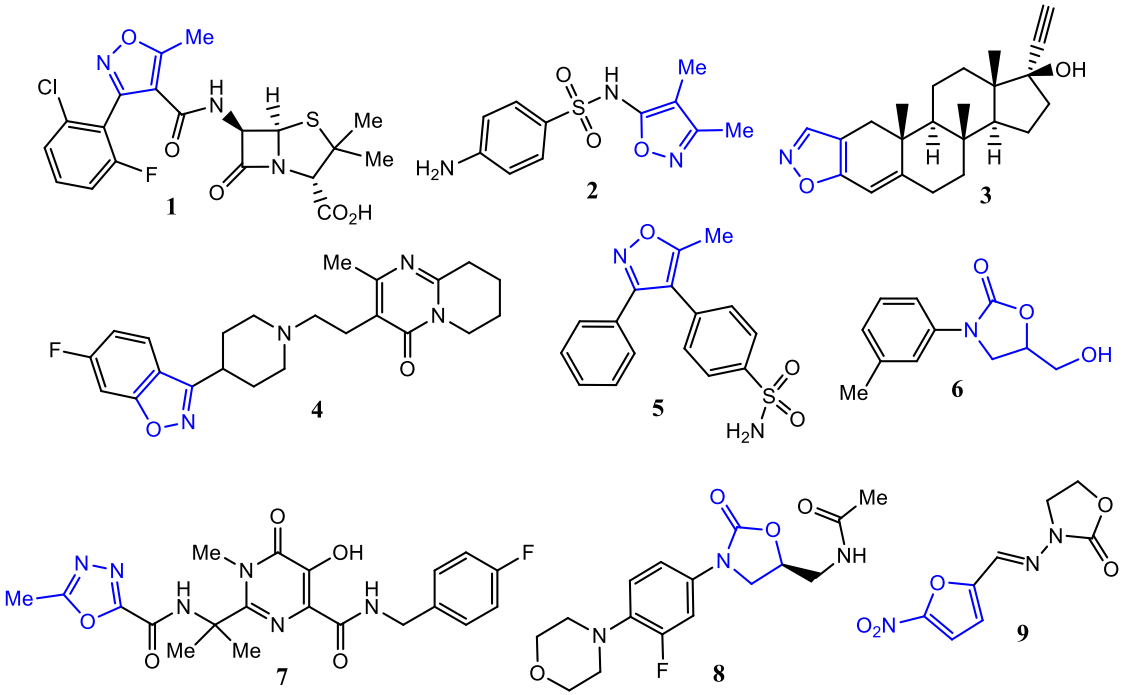


Figure 1. Some structures of oxazole and isoxazole drugs

Up to now, a large number of isoxazole- or oxazole-based medicinal drugs have been extensively used in clinic, such as Flucloxacillin **1**, Sulfisoxazole **2**, Danazol **3**, Risperidone **4**, Valdecoxib **5**, Toloxatone **6**, Raltegravir **7**, Linezolid **8** as well as Furazolidone **9** (Fig. 1). Recently, numerous researchers have been devoting to oxazole compounds as medicinal agents and hopefully discover novel chemical scaffold compounds with broad spectrum, high bioactivity, low toxicity and excellent pharmacokinetic property. Tetrahydropyrrolo-3,4-azoles represent an interesting family of heterocycles that offer potential utility in drug design as either scaffolds or pharmacophoric elements.

Results and discussion

Antiproliferative activity of pyrrolo[3,4-d]isoxazoles against human cervical carcinoma (HeLa) as well as murine fibroblast (3T3) and SV-40 transformed murine fibroblast (3T3-SV40) cell lines was evaluated *in vitro* for 24 and 72 h using the standard MTS assay. Structures of tested compounds are presented at figure 2, while the results of these investigations are presented at figures 3-5.

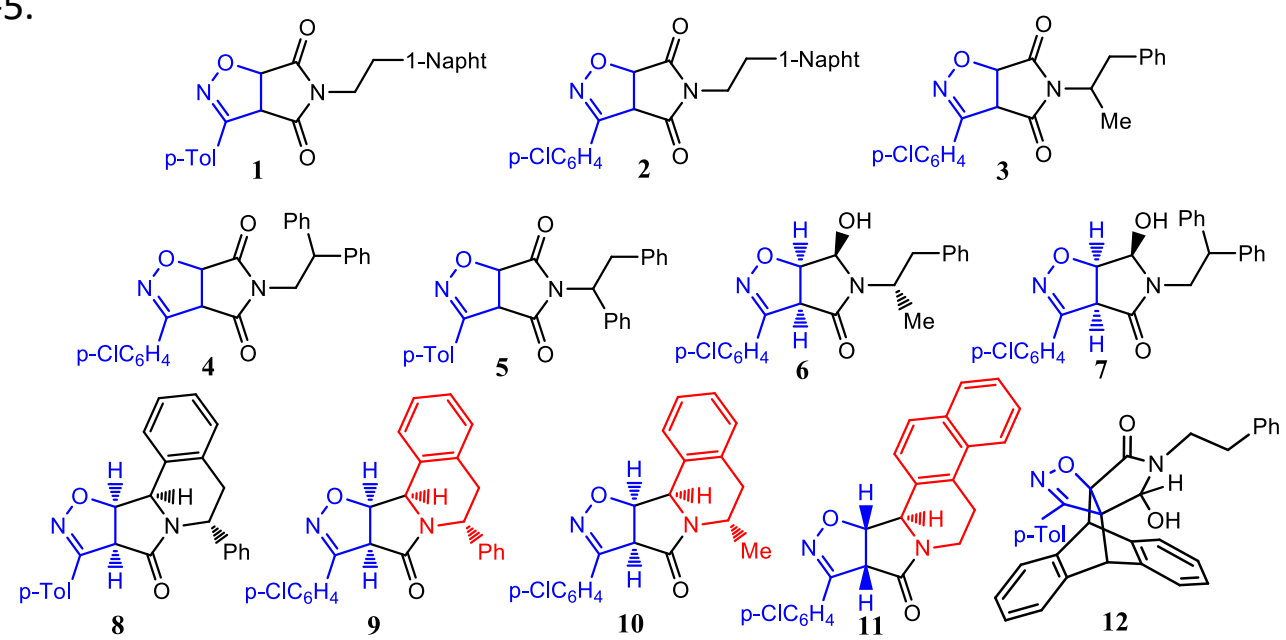


Figure 2. Structures of tested pyrrolo[3,4-d]isoxazole derivatives

Results and discussion

The results showed that adducts **6**, **7** and **11** demonstrated significant activity among the tested compounds, with IC_{50} 14 ± 2, 8 ± 2, 7 ± 2 μg/mL after treatment for 72 h in HeLa (for **6**, **7** and **11** respectively) and 7 ± 2 μg/mL after treatment for 72 h in 3T3-SV40 cell line (for **7**).

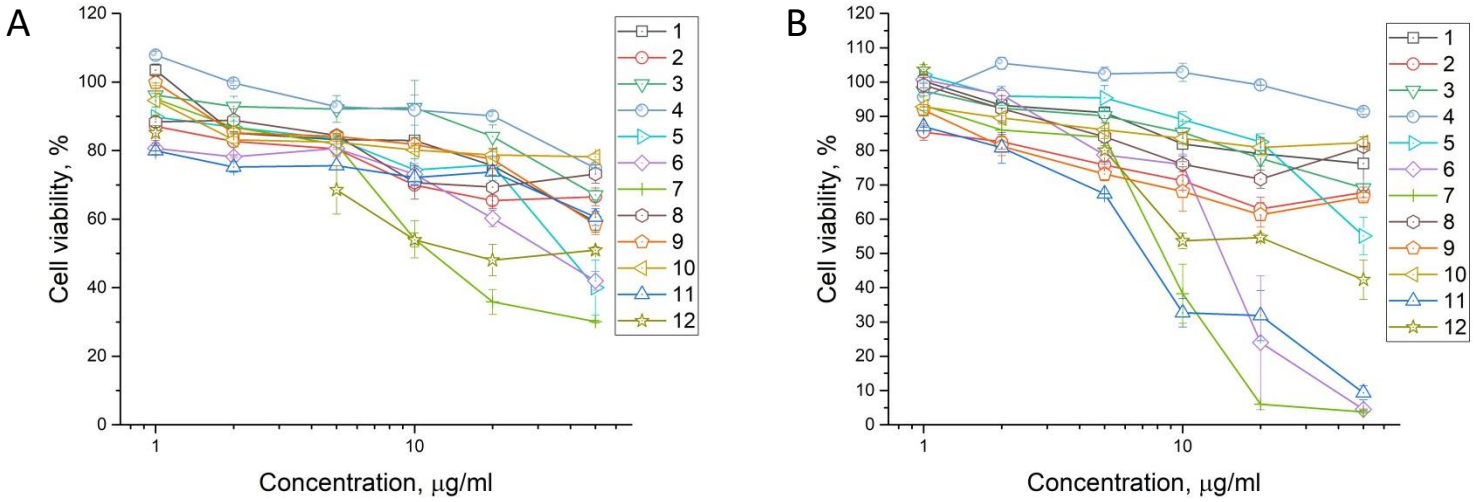


Figure 3. Antiproliferative activity of studied compounds against human cervical carcinoma cell line after 24 (A) and 72h (B)

Results and discussion

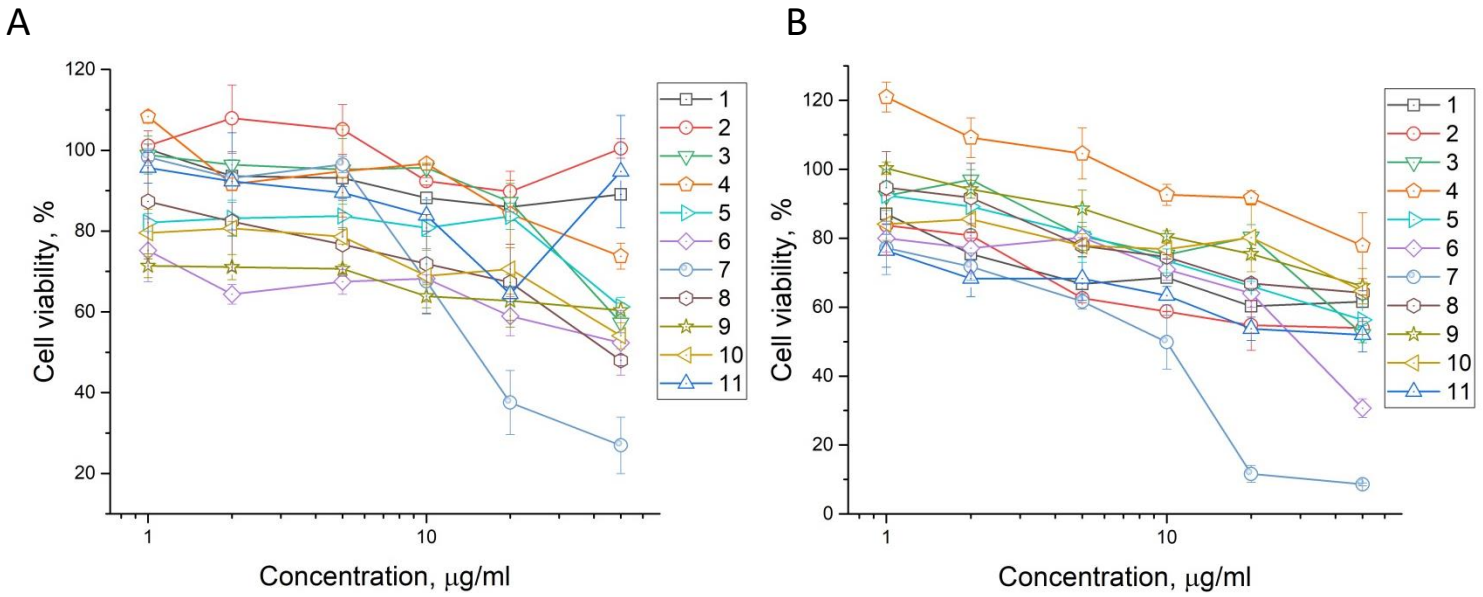


Figure 4. Antiproliferative activity of studied compounds
against SV-40 transformed murine fibroblast 3T3-SV40 cell line after24 (A) and 72h (B)

Results and discussion

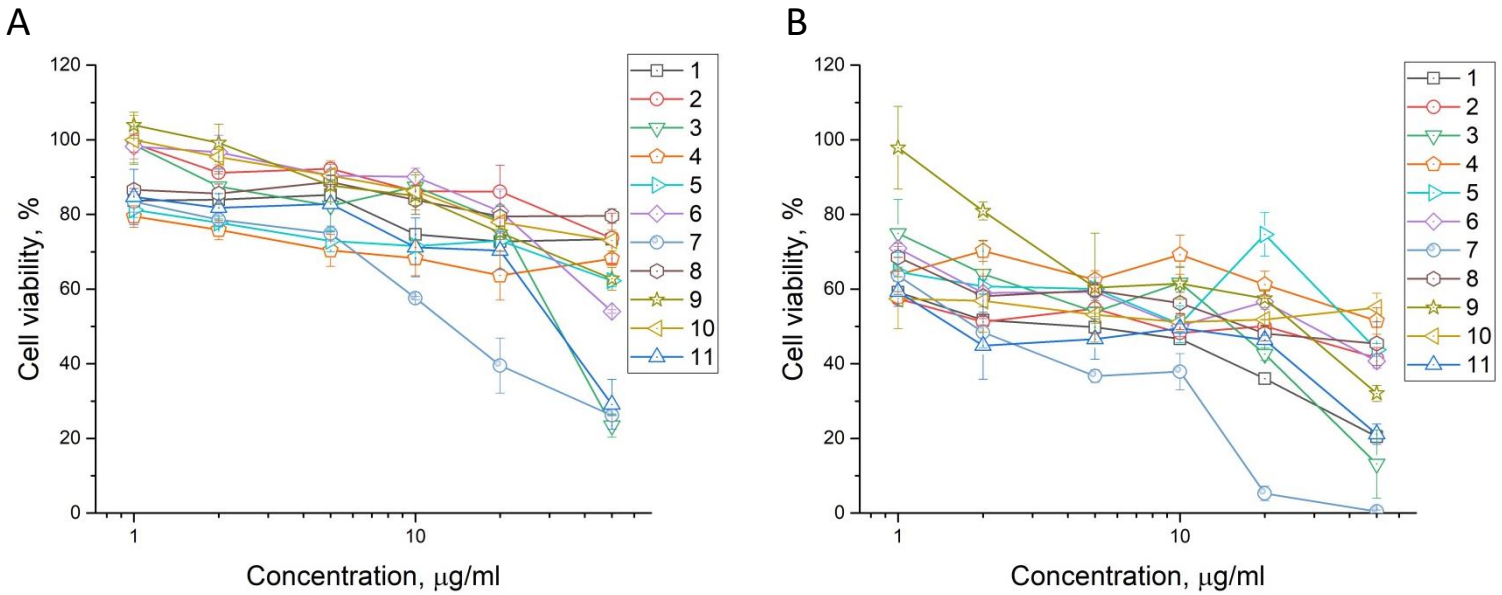


Figure 4. Antiproliferative activity of studied compounds against murine fibroblast 3T3 cell line after24 (A) and 72h (B)

Results and discussion

Actin cytoskeleton structure plays an essential role in vital cellular processes such as cell adhesion, migration, morphogenesis and might be utilized as an additional target for chemotherapeutic intervention. 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.

Based on obtained data compounds **3**, **4**, **6**, **7** and **12** were chosen for additional assessment of the impacts 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 6).

It was found that treatment with antiproliferatively active compound **6** has 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 92% in control to 18%) and stress fibers disappearance (decreases from 84% in control to 29%).

It is interesting to note that even much less antiproliferatively active compounds **3** and **4** have led to meaningful alterations in actin cytoskeleton structure (number of filopodia-like deformations decreases to 44% and 36% while number of stress fibers decreases to 59% and 36% (for **3** and **4** respectively)).

Results and discussion

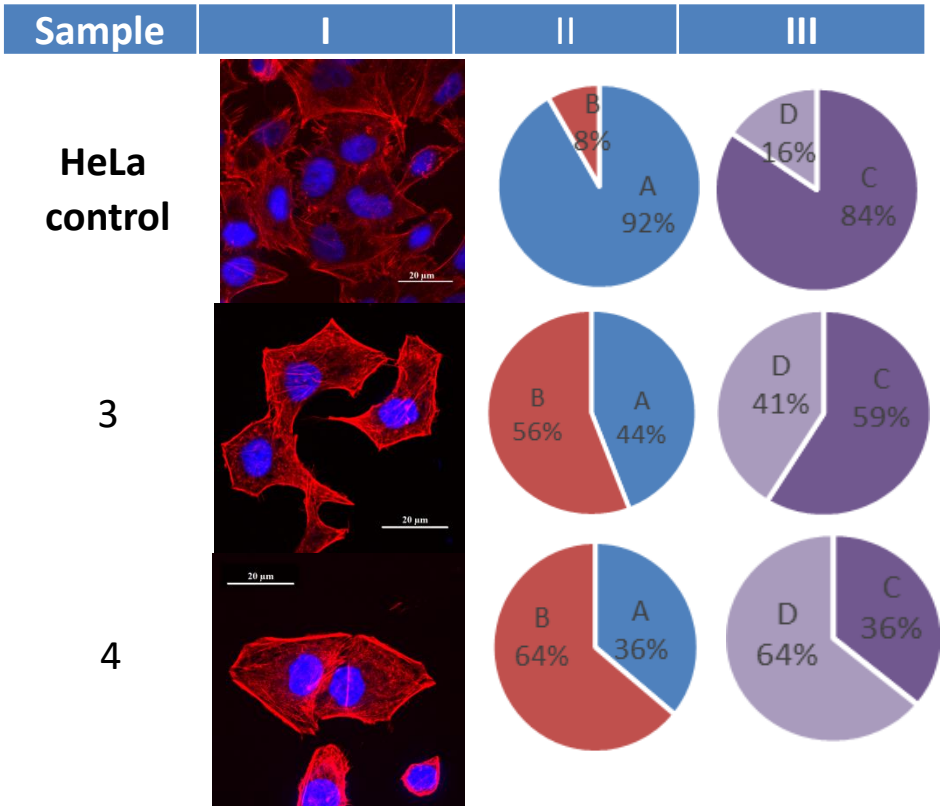


Figure 6. State of actin cytoskeleton of HeLa cells after treatment with pyrrolo[3,4-d]isoxazoles **3**, **4**, **6**, **7** and **12**. I: Images demonstrate the different stages of cell actin cytoskeleton. II: Pie charts demonstrate percentage of cells with filopodia-like deformations (A) and without filopodia-like deformations (B). III: Pie charts demonstrate percentage of cells with normal stress fibers (C) and disassembled stress fibers (D).

Conclusions

Series of pyrrolo[3,4-d]isoxazole derivatives were studied for their antiproliferative activity against used cancer cell lines. Among them, pyrrolo[3,4-d]isoxazoles **6**, **7** and **11** were found to be the most active compound against human cervical carcinoma (HeLa) cell line (IC_{50} 14 ± 2, 8 ± 2 and 7 ± 2 µg/mL after treatment for 72 h, for **6**, **7** and **11** respectively) while pyrrolo[3,4-d]isoxazole **7** was the most active compound against SV-40 transformed murine fibroblast 3T3-SV40 cell line (IC_{50} 7 ± 2 µg/mL after treatment for 72 h). Confocal microscopy revealed that actin filaments disappeared and granular actin was distributed diffusely in the cytoplasm of up to 71% of HeLa cells after their treatment with tested compounds. We showed that the number of HeLa cells with filopodium-like membrane protrusions was reduced significantly (from 92% in control cells to 18%) after treatment with most active compounds. Notably even much less antiproliferative active compounds still have meaningful effect on cytoskeleton structure organization. The obtained experimental data indicate the expediency of searching for pharmacologically active substances among pyrrolo[3,4-d]isoxazole derivatives.

Acknowledgments

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