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SHORT COMMUNICATIONS

In Vitro Activity of Organochalcogen Compounds: II.[1] Cytotoxic Effect of 2-Aminobenzo[*b*]thiophenes Against K562 and HeLa Tumor Cell Lines

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Abstract—In vitro antitumor activity of some benzo[*b*]thiophenes with a tertiary amino group in the second position was studied against erythroleukemia (K562) and cervical carcinoma (HeLa) cell lines.

Keywords: benzo[*b*]thiophenes, 1,2,3-thiadiazoles, in vitro antitumor activity, erythroleukemia (K562) cell line, cervical carcinoma (HeLa) cell line

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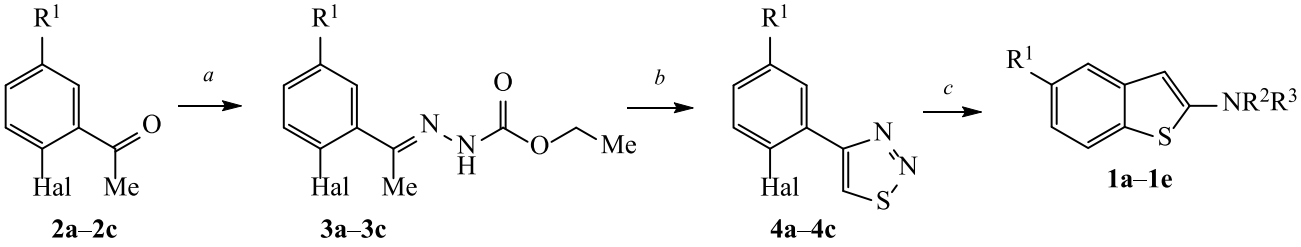
Benzo[*b*]thiophene and its derivatives occupy a special place among sulfur-containing heterocycles, since the compounds containing this fragment have a surprisingly wide spectrum of biological activity [2]. Among the beneficial properties found for benzo[*b*]-thiophene derivatives are anti-cancer [3–8], anti-diabetic [9], anti-tuberculosis [10], anti-malarial [11], anti-fungal [12], anti-depressant [13], anti-convulsant [14], anti-hyperglycemic [15], anti-angiogenic [16], antimitotic [17], anti-inflammatory, and analgesic [18] deserves special attention. Also on the basis of benzo[*b*]thiophene, inhibitors of acetyl-CoA carboxylase [19, 20] and tubulin polymerization [21, 22] were synthesized. It should be noted that 2-aminobenzo[*b*]thiophenes have been successfully used to create selective estrogen receptor modulator (SERM) raloxifene and its analogues [23, 24]. Based on the above information, we have determined the purpose of this work in assessing in vitro antitumor activity of some benzo[*b*]thiophenes, which have a tertiary amino group in the second position. Also we would like to emphasize here that our research group is actively developing original synthetic methods for the preparation of polysubstituted benzothiophenes with amino-, alkoxy- and alkylthio-substituents in the second position based on reactions involving 1,2,3-thiadiazoles [25–29].

In this work, substituted benzo[*b*]thiophenes **1a**–**1e** obtained according to previously developed by us protocols were selected as objects for in vitro study (Fig. 1).

The synthesis of compounds **1a**–**1e** is presented in Scheme 1. Initially, the reaction of acetophenones **2a**–**2c** with ethyl hydrazinecarboxylate yielded the corresponding ethoxycarbonylhydrazones **3a**–**3c**. At the next stage, hydrazones **3a**–**3c** were treated with thionyl chloride to form 4-aryl-1,2,3-thiadiazoles **4a**–**4c** (Hurd–Mori reaction) [30–32]. At the final stage, compounds **4a**, **4b** reacted with various secondary amines such as pyrrolidine, 2-ethylpiperidine, and morpholine in DMF in the presence of copper(I) iodide and potassium carbonate to form 2-aminobenzo[*b*]thiophenes **1a**–**1d** in 60–82% [25]. The interaction of 4-(2-chloro-5-nitrophenyl)-1,2,3-thiadiazole (**4c**) with morpholine in DMF medium at

[1] For communication I, see [1].

Scheme 1. Synthesis of benzo[*b*]thiophenes **1a**–**1e**.



2–4: R^1 = H, Hal = Br (**a**); R^1 = MeO, Hal = Br (**b**); $R^1 = NO_2$, Hal = Cl (**c**). **1**: R^1= H, NR^1R^2 = pyrrolidin-1-yl (**a**); R^1 = H, NR^1R^2 = 2-ethylpiperidin-1-yl (**b**); R^1 = H, NR^1R^2 = morpholin-4-yl (**c**); R^1 = MeO, NR^1R^2 = morpholin-4-yl (**d**); $R^1 = NO_2$, NR^1R^2 = morpholin-4-yl (**e**)

Reaction conditions: *a*, $NH_2NHC(O)OEt$, EtOH, 3 h, 80°C; *b*, $SOCl_2$, 2 h, 80°C; *c*, for **1a**–**1d**: HNR^1R^2, K_2CO_3, CuI (20 mol %), DMF, inert atmosphere, 4–8 h, 80°C, for **1e**: morpholine, DMF, inert atmosphere, 12 h, 80°C.

80°C proceeds without a catalyst to form 4-(4-nitrobenzo[*b*]-thiophen-2- yl)morpholine (**4e**) in 12% yield [32].

Next, the cytotoxic effect of the synthesized 2-aminobenzo[*b*]thiophenes **1a**–**1e** was investigated against human chronic myeloid leukemia (K562) and cervical carcinoma (HeLa) cell lines by MTS or AlamarBlue assay. The results are summarized in Figs. 2, 3. As shown in Figs. 2, 3 one of compounds exhibited potent antiproliferative activity against these two cell lines in an incubation time- and dose-dependent manner. First cytotoxic effects of the 2-substituted benzo[*b*]thiophenes **1a**–**1e** were tested against K562 cell line and then a short test for their activity against HeLa cell line was performed. Among the screened compound **1b** bearing 2-ethylpiperidine substituent showed significant cell growth inhibition against both tumor cell lines (IC_{50} 2 and 10 μg/mL for K562 and HeLa respectively).

In summary, a series of 2-substituted benzo[*b*]thiophenes **1a**–**1e** were prepared and tested for their potent antiproliferative activity against two cancer cell lines. Among them, only 1-(benzo[*b*]thiophen-2-yl)-2-ethylpiperidine (**1b**) was the most promising compound against both K562 and HeLa cell lines tested. The obtained experimental data indicate the expediency of searching for pharmacologically active substances among 2-aminobenzo[*b*]thiophene derivatives.

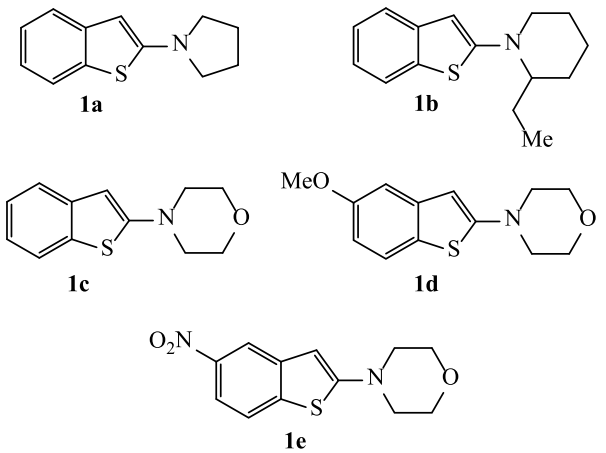


Fig. 1. Structures of benzo[*b*]thiophenes **1a**–**1e.**

Melting points were measured on a Boetius melting point apparatus. ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX-400 spectrometer at 400.13 and 100.16 MHz, respectively. High-resolution mass spectra (ESI) were taken on a Micromass 70-VSE instrument. The reaction progress was monitored by TLC on Silica gel 60 F254 plates; spots were visualized under UV light or by treatment with iodine vapor. The solvents used were purified and dried according to standard procedures. All chemicals were used as purchased.

1-(Benzo[*b*]thiophen-2-yl)pyrrolidine (1a) was prepared in accordance with the developed by us method as described in [25]. Yield 78%.

1-(Benzo[*b*]thiophen-2-yl)-2-ethylpiperidine (1b) was prepared in accordance with the developed by us method as described in [25]. Yield 60%.

4-(Benzo[*b*]thiophen-2-yl)morpholine (1c) was prepared in accordance with the developed by us method as described in [33]. Yield 82%.

Ethyl 2-(1-(2-bromo-5-methoxyphenyl)ethyliden)-hydrazinocarboxylate (3b) was prepared in accordance with the developed by us method as described in [34]. Yield 41.5%.

4-(2-Bromo-5-methoxyphenyl)-1,2,3-thiadiazole (4b). The substance **3b** (3.9 g, 0.013 mol) was placed in a 50 mL two-necked flask equipped with a reflux con-

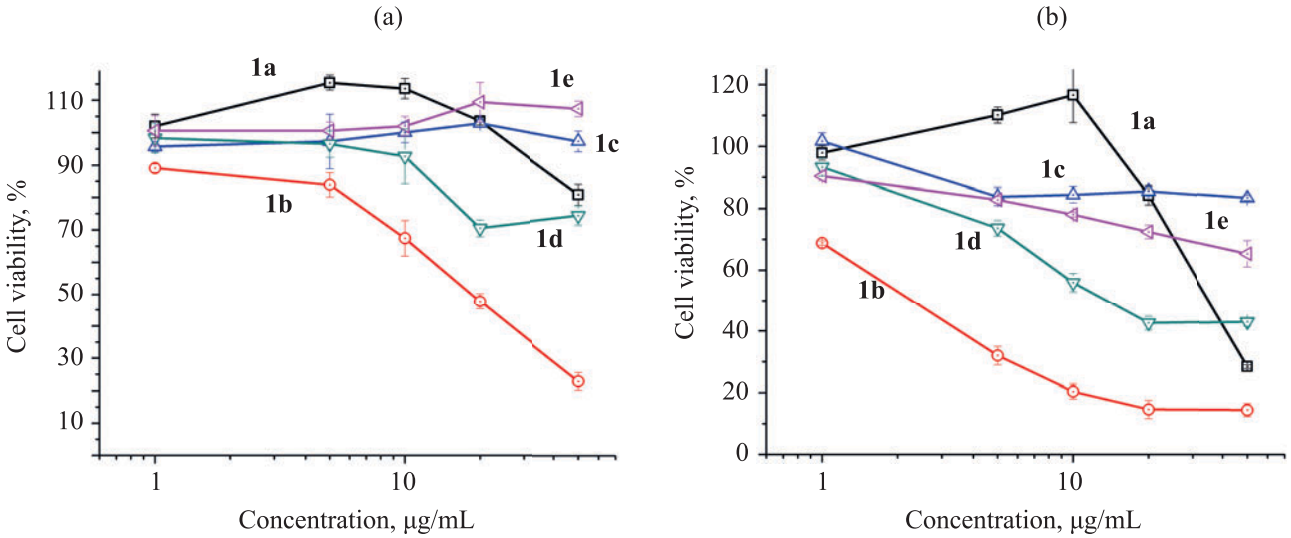


Fig. 2. Cytotoxicity of compounds **1a**–**1e** against the human leukemia K562 cell line (MTS assay): (a) 24 h and (b) 72 h.

denser, magnetic stirrer, calcium chloride tube, and a system for removing gaseous HCl. While cooling to 0–5°C, 20 mL of cooled thionyl chloride was added to **3b**. Then the cooling bath was removed and the reaction mixture was heated to 50°C (until the beginning of the evolution of gaseous HCl). The reaction mixture was stirred with heating for 8 h. Thionyl chloride was evaporated under reduced pressure, the residue was poured into 20 mL of cold water. The formed precipitate was filtered off, washed with water until neutral, and recrystallized from ethanol. Yield 2.05 g (75.6%), mp 118–119°C. ^{1}H NMR spectrum (CDCl3), δ, ppm: 3.88 (3H), 6.91 d.d (1H, J_{meta} = 3.2 Hz, J_{ortho} = 8.8 Hz), 7.57 d (1H, J_{meta} = 3.2 Hz), 7.62 d (1H, J_{ortho} = 8.8 Hz), 9.07 s (1H). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 55.7, 112.5, 116.9, 117.4, 132.2, 134.6, 134.9, 159.1, 160.5. Mass spectrum (HRMS-ESI), *m/z*: 270.9543 $[M + H]^+$ (calculated for $C_{13}H_{15}NO_2S$: 270.9535).

4-(4-Methoxybenzo[*b*]thiophen-2-yl)morpholine (1d). The 1,2,3-thiadiazole **4b** (0.271 g, 1.00 mmol), K_2CO_3 (0.420 g, 3.00 mmol), morpholine (0.175 g, 2.00 mmol), anhydrous copper(II) acetate (0.032 g, 0.20 mmol) and 3 mL of DMF were placed in a 10 mL single-necked round-bottom flask equipped with a magnetic stirrer, reflux condenser and bubble counter. The reaction mixture was degassed three times (3–4 min each, vacuum/argon). The reaction mixture was heated in an argon atmosphere at 80°C for 4 h. The color of the reaction mixture was observed to change from blue-green to yellow. After completion of the reaction, the reaction mixture was cooled and poured into 50 mL of saturated ammonium chloride solution. The resulting solution was extracted with chloroform (3×10 mL), the extract was washed with saturated NaCl solution (2×20 mL), water (2×20 mL), dried over Na_2SO_4, the solvent was removed in vacuo. The residue was purified on SiO_2 column (10×3 cm) (eluent hexane/ethyl acetate, 4 : 1). Yield 0.220 g (88.3%), white solid, mp 118–119°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 3.26 m (2H, J = 4.9 Hz), 3.86 s (3H), 3.88 m (2H, J = 4.9 Hz), 6.19 s (1H), 6.77 d.d (1H, J_{ortho} = 8.8 Hz, J_{meta} = 2.2 Hz), 7.01 d (1H, J = 2.2 Hz), 7.49 d (1H, J = 8.8 Hz). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 50.9, 55.5, 66.3, 99.4, 104.5, 110.6, 122.3, 124.8, 141.4, 157.8, 159.0. Mass spectrum (HRMS-ESI), *m/z*: 234.0907 $[M + H]^+$ (calculated for $C_{13}H_{15}NO_2S$: 250.0896).

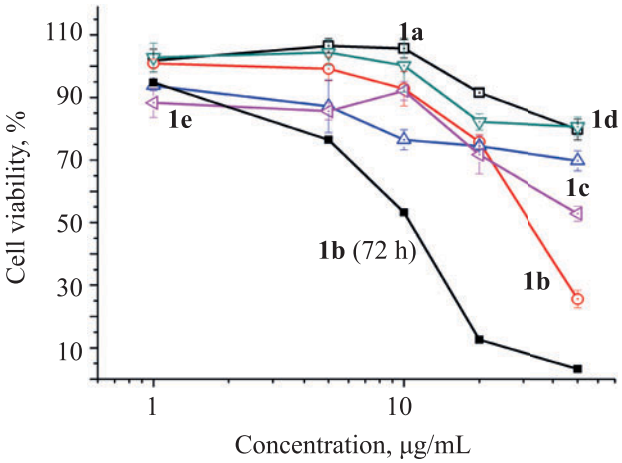


Fig. 3. Cytotoxicity of compounds **1a**–**1e** against the human cervical carcinoma HeLa cell line (AlamarBlue assay): 24 h (**1a**, **1c**–**1d**), 24 and 72 h (**1b**).

4-(4-Nitrobenzo[*b*]thiophen-2-yl)morpholine (1e) was prepared in accordance with the developed by us method as described in [33]. Yield 12 %.

Bioassay details. *Tumor cell line*. The erythroleukemia (K562) and cervical carcinoma (HeLa) cell lines were obtained from the Bank of Cell Cultures of the Institute of Cytology of the Russian Academy of Sciences. K562 cells were cultured in the RPMI-1640 medium (Thermo scientific, USA) with the addition of 10% fetal calf serum (FCS) (Thermo scientific, USA) and 40 μg/mL gentamicin (Sigma, USA). HeLa cells were cultured in the DMEM medium (Thermo scientific, USA) with the addition of 10% fetal calf serum (FCS) (Thermo scientific, USA) and 40 μg/mL gentamicin (Sigma, USA).

MTS assay. A colorimetric MTS assay was used for assessing K562 cell metabolic activity. Shortly, cells were seeded in a 96-well microtiter plates at a density of 100×10^3 cells per well in 100 μL of complete medium and allowed to grow and adhere onto the wells during 24 h at 37°C. After that the cells were treated with various concentrations of the compounds for a period of 24 or 72 h. After the treatment, 20 μL of MTS reagent was added into each well and incubated at 37°C for 2 h. Finally the absorbance was recorded at 490 nm using 96 well plate reader "Multiskan GO" (Thermo Fisher Scientific, USA). For colored solutions the protocol was modified as followed: the absorbance was recorded directly before the addition of MTS reagent and measured values were further subtracted from final absorbance.

AlamarBlue assay. The AlamarBlue Cell Viability Assay Reagent was used to quantify HeLa cellular metabolic activity [35]. The dye incorporates an oxidation-reduction (REDOX) indicator that both fluoresces a4nd change colour in response to the chemical reduction due to cell growth. The alamarBlue dye in its oxidized form is blue in colour and non-fluorescent. In alamarBlue Reagent assay, the growing cells cause a chemical reduction of the alamarBlue dye from non-fluorescent blue to fluorescent red. The continued growth of viable cells maintain a reducing environment (fluorescent, red) and inhibition of growth maintains an oxidized environment (non-fluorescent, blue), which can be detected using a fluorescence or absorbance detector Shortly, cells were seeded in a 96-well microtiter plates at a density of 100×10^3 cells per well in 100 μL of complete medium and allowed to grow and adhere onto the wells during 24 h at 37°C. After that the cells were treated with various concentrations of the compounds for a period of 24 or 72 h. After the treatment, 10 μL of AlamarBlue reagent was added into each well and incubated at 37°C for 2 h. Finally the absorbance was recorded at 570 and 600 nm using 96 well plate reader "Multiskan GO" (Thermo Fisher Scientific, USA). For coloured solutions the protocol was modified as followed: the absorbance was recorded directly before the addition of alamarBlue reagent and measured values were further subtracted from final absorbance.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

1. Remizov, Yu.O., Kornev, A.A., Pevzner, L.M., Petrov, M.L., Boitsov, V.M., and Stepakov, A.V., *Russ. J. Gen. Chem*., 2020, vol. 90, no. 11, p. 2208. https://doi.org/10.1134/S1070363220110328
2. Keri, R.S., Chand, K., Budagumpi, S., Somappa, S.B., Patil, S.A., and Nagaraja, B.M., *Eur. J. Med. Chem.*, 2017, vol. 138, p. 1002. https://doi.org/10.1016/j.ejmech.2017.07.038
3. Martorana, A., Gentile, C., Perricone, U., Piccionello, A.P., Bartolotta, R., Terenzi, A., Pace, A, Mingoia, F., Almerico, A.M., and Lauria, A., *Eur. J. Med. Chem.*, 2015, vol. 90, p. 537. https://doi.org/10.1016/j.ejmech.2014.12.002
4. Arsenyan, P., Paegle, E., Domracheva, I., Gulbe, A., Kanepe-Lapsa, I., and Shestakova, I., *Eur. J. Med. Chem.*, 2014, vol. 87, p. 471. https://doi.org/10.1016/j.ejmech.2014.09.088
5. Arsenyan, P., Paegle, E., Beliakov, S., Shestakova, I., Jaschenko, E., Domracheva, I., and Popelis, J., *Eur. J. Med. Chem*., 2011, vol. 46, p. 3434. https://doi.org/10.1016/j.ejmech.2011.05.008
6. Keri, R.S., Chand, K., Budagumpi, S., Balappa Somappa, S., Patil, S.A., and Mallanna Nagaraja, B., *Eur. J. Med. Chem.*, 2017, vol. 138, p. 1002-1033. https://doi.org/10.1016/j.ejmech.2017.07.038
7. Mhetre, A.B., Lee, H., Yang, H., Lee, K., Namb, Do-H., and Lim, D., *Org. Biomol. Chem.*, 2017, vol. 15, no. 5, p. 1198. https://doi.org/10.1039/C6OB02729F
8. Mániková, D., Šestáková, Z., Rendeková, J., Vlasáková, D., Lukáčová, P., Paegle, E., Arsenyan, P., and Chovanec, M., *Molecules*, 2018, vol. 23, no. 2, p. 507. https://doi.org/10.3390/molecules23020507
9. Moinet, G., Leriche, C., and Kergoat, M., EP1685121 A1. PCT/EP2004/012075.
10. Rao, G.K., and Subramaniam, R., *Chem. Sci. J*., 2015,

vol. 6, p. 92.
https://doi.org/10.4172/2150-3494.100092
11. Banerjee, T., Kapoor, N., Surolia, N., and Surolia, A., *IUBMB Life*, 2011, vol. 63, no. 12, p. 1111. https://doi.org/10.1002/iub.543
12. Jagtap, V.A. and Agasimundin, Y.S., *J. Pharm. Research*, 2015, vol. 9, no. 1, p. 10.
13. Berrade, L., Aisa, B., Ramirez, M.J., Galiano, S., Guccione, S., Moltzau, L.R., Levy, F.O., Nicoletti, F., Battaglia, G., Molinaro, G., Aldana, I., Monge, A., and Perez-Silanes, S., *J. Med. Chem.*, 2011, vol. 54, no. 8, p. 3086. https://doi.org/10.1021/jm2000773
14. Zaher, A.F., Khalil, N.A., and Ahmed, E.M., *Ori. J. Chem*., 2010, vol. 26, no. 4, p. 1241.
15. Malamas, M.S., Sredy, J., Moxham, C., Katz, A., Xu, W., McDevitt, R., Adebayo, F.O., Sawicki, D.R., Seestaller, L., and Sullivan, J.R.D., *J. Med. Chem.*, 2000, vol. 43, no. 7, p. 1293. https://doi.org/10.1021/jm990560c
16. Jeong, S.J., Higuchi, R., Miyamoto, T., Ono, M., Kuwano, M., and Mawatari, S.F., *J. Nat. Prod*., 2002, vol. 65, no. 9, p. 1344. https://doi.org/10.1021/np010577+
17. Romagnoli, R., Baraldi, P.G., Carrion, M.D., Cara, C.L., Preti, D., Fruttarolo, F., Pavani, M.G., Tabrizi, M.A., Tolomeo, M., Grimaudo, S., Di Cristina, A., Balzarini, J., Hadfield, J.A., Brancale, A., and Hamel, E., *J. Med. Chem.*, 2007, vol. 50, no. 9, p. 2273. https://doi.org/10.1021/jm070050f
18. Fakhr, I.M.I., Radwan, M.A.A., El-Batran, S., Abd El-Salam, O.M.E., and El-Shenawy, S.M., *Eur. J. Med. Chem.,* 2009*,* vol. 44, no. 4, p. 1718. https://doi.org/10.1016/j.ejmech.2008.02.034
19. Chonan, T., Wakasusugi, D., Yamamoto, D., Yashiro, M., Oi, T., Tanaka, H., Ohoka-Sugita, A., Io, F., Koretsune, H., and Hiratate, A., *Bioorg. Med. Chem.*, 2011, vol. 19, no. 5, p. 1580. https://doi.org/10.1016/j.bmc.2011.01.041
20. Kamata, M., Yamashita, T., Kina, A., Funata, M., Mizukami, A., Sasaki, M., Tani, A., Funami, M., Amano, N., and Fukatsu, K., *Bioorg. Med. Chem. Lett.*, 2012, vol. 22, no. 11, p. 3643. https://doi.org/10.1016/j.bmcl.2012.04.047
21. Romagnoli, R., Baraldi, P.G., Lopez-Cara, C., Preti, D., Aghazadeh Tabrizi, M., Balzarini, J., Bassetto, M., Brancale, A., Fu, X.-H., Gao, Y., Li, J., Zhang, S.-Z., Hamel, E., Bortolozzi, R., Basso, G., and Viola, G., *J. Med. Chem.*, 2013, vol. 56, no. 22, p. 9296. https://doi.org/10.1021/jm4013938
22. Romagnoli, R., Baraldi, P.G., Carrion, M.D., Cruz-Lopez, O., Tolomeo, M., Grimaudo, S., Cristina, A.D., Pipitone, M.R., Balzarini, J., and Brancale, A., *Bioorg. Med. Chem.*, 2010, vol. 18, no. 14, p. 5114. https://doi.org/10.1016/ j.bmc.2010.05.068
23. Grese, T.A., Cho, S., Finley, D.R., Godfrey, A.G., Jones, C.D., Lugar, C.W., Martin, M.J., Matsumoto, K., Pennington, L.D., and Winter, M.A., *J. Med. Chem.*, 1997, vol. 40, no. 2, p. 146. https://doi.org/10.1021/jm9606352
24. Grese, T.A., Pennington, L.D., Sluka, J.P., Adrian, M.D., Cole, H.W., Fuson, T.R., Magee, D.E., Phillips, D.L., Rowley, E.R., and Shetler, P.K., *J. Med. Chem.*, 1998, vol. 41, no. 8, p. 1272. https://doi.org/10.1021/jm970688z
25. Popova, E.A., Lyapunova, A.G., Petrov, M.L., Panikorovskii, T.L., and Androsov, D.A., *Russ. J. Gen. Chem*., 2018, vol. 88, no. 4, p. 689. https://doi.org/10.1134/S1070363218040126
26. Petrov, M.L., Popova, E.A., and Androsov, D.A., *Russ. J. Org. Chem*., 2015, vol. 51, no. 7, p. 1040. https://doi.org/10.1134/S1070428015070283
27. Androsov, D.A., Popova, E.A., Petrov, M.L., and Ponyaev, A.I., *Russ. J. Gen. Chem*., 2014, vol. 84, no. 12, p. 2405. https://doi.org/10.1134/S1070363214120093
28. Teplyakov, F.S., Vasileva, T.G., Petrov, M.L., and Androsov, D.A. *Org. Lett.*, 2013, vol. 15, no. 15, p. 4038. https://doi.org/10.1021/ol401981w
29. Lyapunova, A.G., Androsov, D.A., and Petrov, M.L., *Tetrahedron Lett*., 2013, vol. 54, no. 26, p. 3427. https://doi.org/10.1016/j.tetlet.2013.04.077
30. 29. Butler, R.N., and O'Donoqhue, D.A., *J. Chem. Soc. Perkin Trans. 1*, 1982, p. 1223. https://doi.org/10.1039/ P19820001223
31. Caplin, A., *J. Chem. Soc. Perkin Trans. 1*, 1974, p. 30. https://doi.org/10.1039/P19740000030
32. Hurd, C.D. and Mori, R.I., *J. Am. Chem. Soc.*, 1955, vol. 77, no. 20, p. 5359. https://doi.org/10.1021/ja01625a047
33. Androsov, D.A., Solovyev, A.Y., Petrov, M.L., Butcher, R., and Jasinski, J., *Tetrahedron*, 2010, vol. 66, no. 13, p. 2474. https://doi.org/10.1016/j.tet.2010. 01.069
34. Popova, E.A., Stepanova, O.V., Bessonov, V.V., Nepochatyi, G.D., Pevzner, L.M., and Petrov, M.L., *Russ. J. Gen. Chem*., 2020, vol. 90, no. 2, p. 311. https://doi.org/10.1134/S1070363220020243
35. The tests were carried out according to manufacturer protocol: https://www.gbiosciences.com/Bioassays/Alamar-Blue-Cell-Viability-Assay.