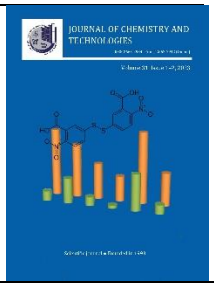
Journal of Chemistry and Technologies, 2023, *31*(1), 1-8

Journal of Chemistry and Technologies

pISSN 2663-2934 (Print), ISSN 2663-2942 (Online).

journal homepage: http://chemistry.dnu.dp.ua



UDC 547.735

SYNTHESIS OF NEW SUBSTITUTED 3-(1,2,4-OXADIAZOL-5-YL)-THIENO[3,2-*E*][1,2,3]TRIAZOLO[1,5-*A*]PYRIMIDIN-5(*4H*)-ONES VIA AZIDE DOMINO REACTIONS

Olga Ya. Shyyka, Mykola A. Tupychak*, Nazariy T. Pokhodylo

[1]*Ivan Franko National University of Lviv, Kyryla and Mefodiya Str., 6, 79005 Lviv, Ukraine*

Received 3 February 2023; accepted 23 February 2023; available online 25 April 2023

Abstract

Fused polyheterocyclic compounds related to thieno[2,3-d]pyrimidines are a widely-used class of heterocycles in medicinal chemistry and have attracted considerable interest as potential anticancer agents. In the current article, the substituted (1,2,4-oxadiazol-5-yl)acetonitriles were implemented in convenient domino reactions with 2-azidothiophene-3-carboxylates for the straightforward synthesis of 3-(1,2,4-oxadiazol-5-yl)-thieno[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidin-5(*4H*)-ones. The 1,2,4-oxadiazole motif was selected to be conjugated with thieno[3,2-e][1,2,3]triazolo[1,5-a]pyrimidine core because several (1,2,4-oxadiazol-5-yl)-1H-1,2,3-triazol-5-amines recently was found as promising antitrypanosomal activity, which is often tied to cytotoxicity against cancer cells. The reaction proceeds at room temperature in a short time with base catalysis and no chromatographic purification of products is required. High purity products were isolated by simple filtration. The synthesized compounds were screened for anticancer activity in the 60 cancer cell panel in NCI. In general, selected 3-(1,2,4-oxadiazol-5-yl)thieno[3,2-e][1,2,3]triazolo[1,5-a]pyrimidin-5(4H)-ones did not show significant antitumor activity. The highest activity was demonstrated by compound 3b, which inhibited the growth of 27% of LOX IMVI melanoma cells at a concentration of 10^{-5} M.

Keywords: thieno[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine; (1,2,4-oxadiazol-5-yl)acetonitriles; azide; domino reaction; anticancer activity

СИНТЕЗ НОВИХ ЗАМІЩЕНИХ 3-(1,2,4-ОКСАДІАЗОЛ-5-ІЛ)-ТІЄНО[3,2-*E*][1,2,3]ТРИАЗОЛО[1,5-*A*]ПІРИМІДИН-5(4*H*)-ОНІВ В ДОМІНО РЕАКЦІЇ АЗИДІВ

Ольга Я. Шийка, Микола А. Тупичак*, Назарій Т. Походило

Львівський національний університет імені Івана Франка, вул. Кирила та Мефодія, 6, 79005 Львів, Україна

Анотація

Конденсовані полігетероциклічні сполуки, пов'язані з тієно[2,3-d]піримідинами, є широко використовуваним класом гетероциклів у медичній хімії та викликають значний інтерес як потенційні протипухлинні засоби. У цій статті, заміщені (1,2,4-оксадіазол-5-іл)ацетонітрили були введені в зручну доміно реакцію з 2-азидотіофен-3-карбоксилатами для прямого синтезу 3-(1,2,4-оксадіазол-5-іл)-тієно[3,2-*e*][1,2,3]триазоло[1,5-*a*]піримідин-5(*4H*)-онів. Мотив 1,2,4-оксадіазолу було обрано для кон'югації з тієно[3,2-e][1,2,3]триазоло[1,5-a]піримідиновим ядром, оскільки, нещодавно було виявлено декілька 4-(1,2,4-оксадіазол-5-іл)-1H-1,2,3-тріазол-5-амінів, що мають багатообіцяючу антитрипаносомну активність, яка часто пов'язана з цитотоксичністю проти ракових клітин. Реакція проходить швидко при кімнатній температурі за основного каталізу і не вимагає хроматографічної очистки продуктів. Продукти високої чистоти виділяли простим фільтруванням. Синтезовані сполуки перевіряли на протипухлинну активність на панелі 60 ракових клітин в NCI. Загалом відібрані 3-(1,2,4-оксадіазол-5-іл)тієно[3,2-e][1,2,3]триазоло[1,5-a]піримідин-5(4H)-они не виявили значної протипухлинної активності. Найбільшу активність виявила сполука 3b, яка пригнічувала ріст 27 % клітин меланоми LOX IMVI у концентрації 10^{-5} М.

Ключові слова: тієно[3,2-*e*][1,2,3]триазоло[1,5-*a*]піримідин; (1,2,4-оксадіазол-5-іл)ацетонітрили; азид; доміно реакція; протипухлинна активність

**Corresponding author: e-mail address: tupychakmykola@gmail.com*

doi: 10.15421/jchemtech.v31i1.273476

Introduction

Fused polyheterocyclic compounds related to thieno[2,3-*d*]pyrimidines have many applications in various fields such as pharmaceutical and materials science due to their unique structural features and similarity with natural purine bases [1; 2]. Many compounds having thieno[2,3-*d*]pyrimidine core exhibit a wide range of important therapeutic activities (Fig 1): for example, pyrido[1,2-a]thieno[3,2-*e*]pyrimidine **A** is an antimicrobial agent acting toward multidrug-resistant *A. baumannii* [3], thieno[3',2':5,6] pyrimido[1,2-*b*]isoquinoline **B** exhibits antitumor moderate activity toward HePG-2 and MCF-7 cell lines [4], pyrazolo[1,5-*a*]thieno[3,2-*e*]pyrimidine **C** is an anticancer agent which acts via inhibiting lactate dehydrogenase A (LDHA) and/ or lactate dehydrogenase B (LDHB) activity in a cell [5]. A number of thieno[2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine derivatives of type **D** were found useful for treating neurodegenerative diseases [6]. Moreover, isomeric sulfonyl-thieno [2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine derivatives have been identified as potent HIV-1 replication inhibitors. [7] In addition, the annelated thieno[3,2-*e*]pyrimidines have significant prospects as anticancer agents [See 8, 9 and article cited therein]. In our recent work, we discovered a thieno[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine **E** with selective influence and high cytotoxicity against melanoma SK-MEL-5 cell line [10]. It is of note that triazolopyrimidines, as analogues of purine bases, are also frequent targets for various biological studies, as evidenced by recent reviews [11; 12].

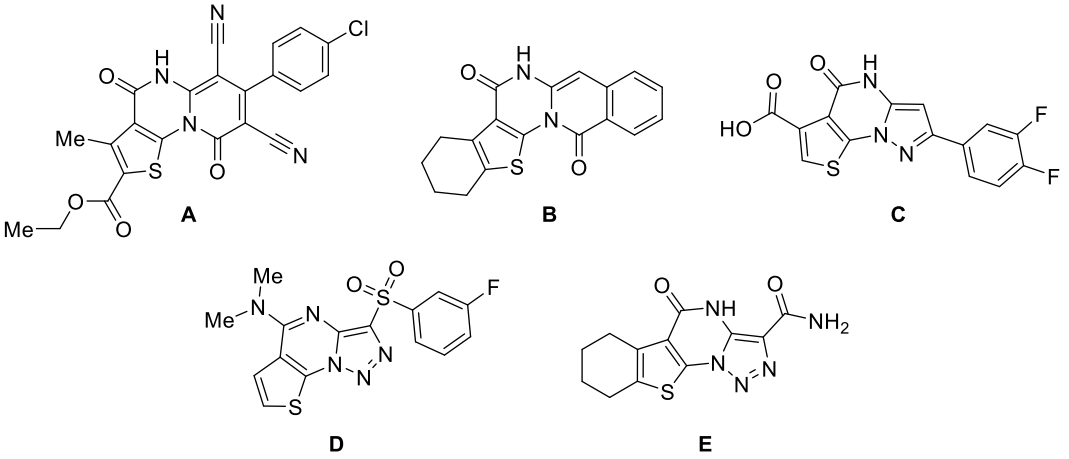


Fig. 1. Biologically active polycyclic thieno[2,3-*d*]pyrimidines

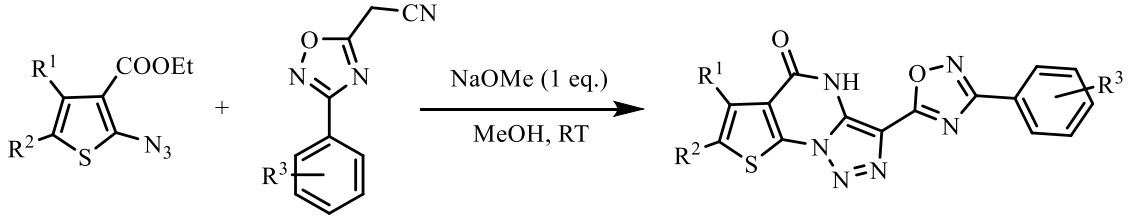
Keeping the importance of condensed thienotriazolopyrimidines, our present studies focused on the implementation of reagents in convenient domino reactions for the synthesis of new thieno[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidines assuming that these compounds may possess remarkable biological activities. The 1,2,4-oxadiazole motif was selected to be conjugated with thieno[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine core because (1,2,4-oxadiazol-5-yl)-*1H*-1,2,3-triazol-5-amines were found as promising agents against the *T. cruzi* parasite (*Trypanosoma cruzi*) [13]. Moreover, recently 1,2,4-oxadiazoles were introduced into several therapeutic molecules currently in focus [14-17]. Therefore, the preparation of a new library of novel thieno [3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidines with 1,2,4-oxadiazole ring via azide domino reaction is inquired.

Results and discussion

Chemistry. The azide domino reactions are convenient and effective for the diversity-oriented synthesis of condensed *1H*-1,2,3-triazole compounds [18]. The advantage of such reactions is high selectivity, ease of execution, reduction of the number of intermediate stages, high economy of atoms. There are many environmental benign protocols producing excellent yields in mild reaction conditions and require short reaction timings, operationally simple medium. Noteworthy, that the use of heterocyclic azides in domino reactions made it possible to obtain new condensed 1,2,3-triazolo[1,5-*a*]pyrimidine systems, such as 1,2,3-triazolo[1,5-*a*]thieno-[3,2-d]pyrimidine [19–22], pyrido[3',2':4,5]-thieno[2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine [23], pyrrolo[3,4-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine [24; 25], indolo[3,2-*e*][1,2,3]triazolo-[1,5-*a*]pyrimidine [26], indolo[2,3-*e*][1,2,3]-triazolo[1,5-*a*]pyrimidine [27; 28], pyrazolo-[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine [29; 30], *8H*-[1,2,3]triazolo[5,1-*b*]purine [31] and triazolo[4,3-*e*]triazolo[1,5-*a*]pyrimidine [32–34]. We used the azido domino reactions for the synthesize thieno[2,3-*e*][1,2,3]triazolo[1,5-*a*]-

pyrimidines with a wide range of substituents [35], investigated the impact of carbonyl substituents in the formation of the pyrimidine ring [36], as well as the direction of such reactions in the case of multifunctional compounds with the possibility of side processes [37; 38]. Thus, the development of new and expanding the limits of application of azide domino reactions is an urgent task.

In order to expand the combinatorial library of thieno[2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidines by introducing the 1,2,4-oxadiazole moiety, we developed a domino reaction in which 2-(3-aryl-1,2,4-oxadiazol-5-yl)acetonitriles react with ethyl 2-azidothiophene-3-carboxylates. The presence of ester groups in the *ortho*-position leads to the spontaneous cyclization of the 5-aminotriazole formed as a result of 1,3-dipolar cycloaddition with the formation of the triazolopyrimidine system. Starting 2-(3-aryl-1,2,4-oxadiazol-5-yl)acetonitriles **1a-e** were obtained by the interaction of aromatic amidoximes with 3-(3,5-dimethyl-*1H*-pyrazol-1-yl)-3-oxopropanenitrile in dioxane with good yields [13]. 2-Azidothiophene-3-carboxylates were obtained from the corresponding 2-aminothiophenes Gewald's. These azides react with 2-(3-aryl-1,2,4-oxadiazol-5-yl)acetonitriles in methanol solution in the presence of one equivalent of sodium methylate at room temperature, which allowed for the first time to obtain 3-(3-aryl-1,2,4-oxadiazol-5-yl)thieno[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidin-5(*4H*)-ones **3** with excellent yields (Table 1). The target products were isolated from the reaction mixture in pure form of white powders by filtration without further purification, consistent with the concepts of click chemistry and green chemistry. The total reaction time was 5 min. The reaction proceeded on the domino principle: in the first stage there is a 1,3-dipolar cycloaddition with the formation of 5-amino-1,2,3-triazoles, which undergo spontaneous intramolecular cyclization with the participation of ester and amino groups with the formation of the pyrimidine ring.



1a,b **2a-e** **3a-h**

Scheme 1. Synthesis of thieno[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidin-5(*4H*)-ones 3a-h

Table 1.

Synthesis of thieno[3,2-e][1,2,3]triazolo[1,5-a]pyrimidin-5(4H)-ones 3a-h

Compound	R^1	R^2	R^3	Yield, %
3a	Me	Me	H	90
3b	Me	Me	2-Me	94
3c	Me	Me	2-Cl	85
3d	Me	Me	4-F	86
3e	Me	Me	4-Cl	83
3f	-$(CH_2)_4$-		2-Cl	69
3g	-$(CH_2)_4$-		4-F	74
3h	-$(CH_2)_4$-		4-Cl	72

The obtained products **3a-h** were then characterized by spectral techniques (^{1}H and ^{13}C NMR). For instance, four signals of the carbons of the thiophene ring at 110.98-111.99, 122.50-125.78, 127.71-130.14 and 152.86-153.20 ppm appeared in the ^{13}C NMR spectra. The C=O group of the pyrimidine ring – at 166.92-167.24 ppm, and the signals of the carbons of the triazole ring appeared at 116.45-116.70 ppm for C-4 atoms and 144.95-145.15 ppm for C-5 atoms. Two groups of oxadiazole ring signals appeared at 166.31-166.87 ppm for C-3 atoms and 169.92-171.04 ppm for C-5 atoms.

Biological activity. Anticancer screening. The anticancer activity of the obtained thieno-[3,2-*e*][1,2,3]-triazolo[1,5-*a*]pyrimidinones **3a-h** was studied within the framework of the international scientific program of the US National Institutes of Health DTP (Developmental Therapeutic Program) of the National Cancer Institute (NCI, Bethesda, Maryland, USA) (http://dtp.nci.nih.gov) [40].

For the synthesized compounds **3a-h**, anticancer activity *in vitro* was studied on 60 cancer cell lines covering almost the entire spectrum of human cancers (lung, breast, ovarian, leukemia, colon, kidney, melanoma, prostate, and central nervous system cancer lines) under the

action of the substance in a concentration of 10^{-5} mol/L, as a result of which the percentage of cell growth of cancer lines was determined in comparison with the control. The procedure for determining antitumor activity was carried out in accordance with the NCI protocol described in [41–43]. Table 2 shows the results of the experiment for the cancer lines that were sensitive to the compounds. The percentage of cell growth (GP, %) was determined for each compound. In general, selected representatives of this molecular scaffold did not show significant antitumor activity. The highest activity was demonstrated by compound **3b**, which inhibited the growth of 27% (GP = 73.16 %) of LOX IMVI melanoma cells. It is worth noting that LOX IMVI melanoma cells were the most sensitive to the action of the studied compounds. Thus, compounds **3a**, **3f**, and **3h** also showed activity, inhibiting their growth at the level of 20% (GP = 76.74, 81.17, and 80.87 %, respectively). In addition, to compound **3f** was the most active against central nervous system cancer cells SNB-75 (GP = 79.01 %) and kidney cancer cells UO-31 (GP = 80.24 %). All investigated thieno[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidines **3a-h** were resistant to a panel of ovarian cancer cells. Complete result of the NCI screen (one dose data) could be found in Supplementary Part.

Table 2.

Anticancer screening data of 3-(3-aryl-1,2,4-oxadiazol-5-yl)thieno-[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine-5(4H)-ones 3a-h at a concentration of 10^{-5} M

	The percentage of cell growth (GP, %) after compounds treatment							
	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**
Leukemia								
SR	89.22	87.77	88.74	97.60	92.90	99.40	99.66	99.81
Non-small cell lung cancer								
EKVX	104.90	92.96	97.04	95.18	91.17	88.03	100.67	95.95
Colon cancer								
HCT-15	95.42	90.33	101.09	92.46	94.36	96.94	87.21	98.15
CNS cancer								
SNB-75	91.41	104.50	96.63	85.72	96.11	**79.01**	93.19	87.89
Melanoma								
LOX IMVI	**76.74**	**73.16**	95.31	93.74	115.97	**81.17**	105.38	**80.87**
Kidney cancer								
CAKI-1	93.07	95.89	95.56	92.78	97.65	86.56	98.00	93.88
UO-31	85.68	90.04	91.64	88.09	91.05	**80.24**	92.49	85.45
Prostate cancer								
PC-3	99.43	93.44	95.99	89.61	100.22	91.92	101.62	93.28
Breast cancer								
MCF7	90.63	91.72	92.79	89.72	90.45	90.36	91.53	87.77
T-47D	99.92	101.22	97.15	95.49	87.66	100.13	91.55	93.28

In general, the studied thieno-[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidin-5(*4H*)-ones **3a-h** did not show significant antitumor activity, which can probably be caused by poor internal penetration cells due to low solubility. This class of compounds requires significant structural modification for further studies of anticancer activity.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded on Varian Unity Plus 400 (400 and 101 MHz, respectively) and Bruker 170 Avance 500 (500 and 126 MHz, respectively) spectrometers in DMSO-d_6 solutions using TMS or the deuterated solvent as internal reference. Mass spectral analyses were performed using an Agilent 1100 series LC/MSD with API-ES/APCI mode (200 eV). Elemental analyses were accomplished using a Carlo Erba 1106 instrument. Melting points were determined on a Boetius melting point apparatus.

General method of synthesis of thieno[3,2-e]-[1,2,3]triazolo[1,5-a]pyrimidin-5(4H)-ones 3a-h

Substituted acetonitrile **2** (0.001 mol) was added to a solution of sodium methylate (0.054 g, 0.001 mol) in dry methanol (20 mL). A solution of 2-azidothiophene **1** (0.001 mol) in dry methanol (2 ml) was added dropwise to this solution. The mixture is stirred for 5 minutes. Control of the reaction is carried out using TLC. The resulting suspension was filtered, and the solid product was washed with water and MeOH to give the target thieno[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidines **3**.

6,7-dimethyl-3-(3-phenyl-1,2,4-oxadiazol-5-yl)-thieno[3,2-e][1,2,3]triazolo[1,5-a]pyrimidin-5(4H)-one 3a: Yield 90 %; brown solid; m.p.: > 300ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (dd, *J* = 6.5, 3.0 Hz, 2H, H^{Ph}-3,5), 7.64 – 7.55 (m, 3H, H^{Ph}-2,4,6), 2.45 (s, 3H, Me), 2.30 (s, 3H, Me). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.90 (C^{oxadiazole}-5), 168.05 (CO), 166.34 (C^{oxadiazole}-3), 153.20 (CTh-3), 144.97

(C^{Tr}-5), 131.72 (CH^{Ph}-4), 129.62 (2xCH^{Ph}-3,5), 127.73 (CTh-5), 127.63 (2xCH^{Ph}-2,6), 127.37 (C^{Ph}-1), 122.66 (CTh-4), 116.70 (C^{Tr}-4), 111.93 (CTh-2), 13.89 (Me), 13.20 (Me). MS (m/z): 365 (M^++1). Anal. calcd. for $C_{17}H_{12}N_6O_2S$: C, 56.04; H, 3.32; N, 23.06; found: C, 56.05; H, 3.30; N, 23.04.

6,7-dimethyl-3-(3-(o-tolyl)-1,2,4-oxadiazol-5-yl)thieno[3,2-e][1,2,3]triazolo[1,5-a]pyrimidin-5(4H)-one 3b: Yield 94 %; orange solid; m.p.: > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (d, J = 7.5 Hz, 1H, H^{Ar}-6), 7.50 – 7.38 (m, 3H, H^{Ar}-3,4,5), 2.61 (s, 3H, Me), 2.45 (s, 3H, Me), 2.30 (s, 3H, Me). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.92 (C^{oxadiazole}-5), 168.64 (CO), 166.31 (C^{oxadiazole}-3), 153.18 (CTh-3), 145.04 (C^{Tr}-5), 137.89 (C^{Tol}-2), 131.76 (CH^{Tol}-3), 130.99 (CH^{Tol}-6), 130.34 (CH^{Tol}-4), 127.74 (CTh-5), 126.82 (C^{Tol}-1), 126.63 (CH^{Tol}-5), 122.50 (CTh-4), 116.61 (C^{Tr}-4), 111.86 (CTh-2), 22.12 (Me), 13.90 (Me), 13.21 (Me). MS (m/z): 379 (M^++1). Anal. calcd. for $C_{18}H_{14}N_6O_2S$: C, 57.13; H, 3.73; N, 22.21; found: C, 57.15; H, 3.71; N, 22.20.

3-(3-(2-chlorophenyl)-1,2,4-oxadiazol-5-yl)-6,7-dimethylthieno[3,2-e][1,2,3]triazolo[1,5-a]pyrimidin-5(4H)-one 3c: Yield 85 %; yellow solid; m.p.: > 300ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99 (dd, J = 7.5, 1.9 Hz, 1H, H^{Ar}-6), 7.69 (d, J = 7.7 Hz, 1H, H^{Ar}-3), 7.66 – 7.52 (m, 2H, H^{Ar}-4,5), 2.45 (s, 3H, Me), 2.29 (s, 3H, Me). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.37 (C^{oxadiazole}-5), 166.93 (CO), 166.32 (C^{oxadiazole}-3), 153.17 (CTh-3), 145.03 (C^{Tr}-5), 132.71 (CH^{Ar}-6), 132.69 (C^{Ar}-2), 132.34 (CH^{Ar}-6), 131.08 (CH^{Ar}-4), 128.05(CH^{Ar}-5), 127.74 (CTh-5), 126.87 (C^{Ar}-1), 122.65 (CTh-4), 116.45 (C^{Tr}-4), 111.97 (CTh-2), 13.89 (Me), 13.20 (Me). MS (m/z): 399 (M^++1). Anal. calcd. for $C_{17}H_{11}ClN_6O_2S$: C, 51.20; H, 2.78; N, 21.07; found: C, 51.09; H, 2.71; N, 21.18.

3-(3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-6,7-dimethylthieno[3,2-e][1,2,3]triazolo[1,5-a]pyrimidin-5(4H)-one 3d: Yield 86 %; brown solid; m.p.: 281-283ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (q, J = 7.1, 6.0 Hz, 2H, H^{Ar}-2,6), 7.41 (t, J = 8.3 Hz, 2H, H^{Ar}-3,5), 2.44 (s, 3H, Me), 2.29 (s, 3H, Me). ^{13}C NMR (126 MHz, DMSO) δ 170.95 (C^{oxadiazole}-5), 167.24 (CO), 166.35 (C^{oxadiazole}-3), 164.29 (d, $^1J_{C\text{-}F}$ = 249.1 Hz, C^{Ar}-4), 153.20 (CTh-3), 144.95 (C^{Tr}-5), 130.11 (d, $^3J_{C\text{-}F}$ = 8.9 Hz, 2xCH^{Ar}-2,6), 127.71 (CTh-5), 123.93 (CTh-4), 123.92 (d, $^4J_{C\text{-}F}$ = 2.5 Hz, C^{Ar}-1), 116.64 (C^{Tr}-4), 116.71 (d, $^2J_{C\text{-}F}$ = 22.0 Hz, 2xCH^{Ar}-3,5), 111.96 (CTh-2), 13.86 (Me), 13.17 (Me). MS (m/z): 383 (M^++1). Anal. calcd. for $C_{17}H_{11}FN_6O_2S$: C, 53.40; H, 2.90; N, 21.98; found: C, 53.28; H, 2.97; N, 21.77.

3-(3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl)-6,7-dimethylthieno[3,2-e][1,2,3]triazolo[1,5-a]pyrimidin-5(4H)-one 3e: Yield 83 %; brown solid; m.p.: > 300ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (d, J = 7.9 Hz, 2H, H^{Ar}-3,5), 7.64 (d, J = 8.2 Hz, 2H, H^{Ar}-2,6), 2.44 (s, 3H, Me), 2.29 (s, 3H, Me). ^{13}C NMR (126 MHz, DMSO) δ 171.04 (C^{oxadiazole}-5), 167.23 (CO), 166.31 (C^{oxadiazole}-3), 153.16 (CTh-3), 145.00 (C^{Tr}-5), 136.40 (C^{Ar}-4), 129.75 (2xCH^{Ar}-3,5), 129.41 (2xCH^{Ar}-2,6), 127.75 (CTh-5), 126.23 (C^{Ar}-1), 122.64 (CTh-4), 116.57 (C^{Tr}-4), 111.99 (CTh-2), 13.88 (Me), 13.19 (Me). MS (m/z): 399 (M^++1). Anal. calcd. for $C_{17}H_{11}ClN_6O_2S$: C, 51.20; H, 2.78; N, 21.07; found: C, 51.02; H, 2.87; N, 21.18.

3-(3-(2-chlorophenyl)-1,2,4-oxadiazol-5-yl)-6,7,8,9-tetrahydrobenzo[4,5]thieno[3,2-e][1,2,3]triazolo[1,5-a]pyrimidin-5(4H)-one 3f: Yield 69 %; yellow solid; m.p.: > 300ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 – 7.95 (m, 1H, H^{Ar}-6), 7.69 (d, J = 7.8 Hz, 1H, H^{Ar}-3), 7.64 – 7.53 (m, 2H, H^{Ar}-4,5), 2.95 (s, 2H, CH$_2$), 2.66 (s, 2H, CH$_2$), 1.80 (s, 4H, 2xCH$_2$). ^{13}C NMR (101 MHz, DMSO) δ 170.39 (C^{oxadiazole}-5), 166.92 (CO), 166.85 (C^{oxadiazole}-3), 152.86 (CTh-3), 145.15 (C^{Tr}-5), 132.71 (CH^{Ar}-6), 132.69 (C^{Ar}-2), 132.35 (CH^{Ar}-6), 131.08 (CH^{Ar}-4), 130.14 (CTh-5), 128.05 (CH^{Ar}-5), 126.89 (C^{Ar}-1), 125.69 (CTh-4), 116.49 (C^{Tr}-4), 110.99 (CTh-2), 26.26 (CH$_2$), 25.16 (CH$_2$), 23.22 (CH$_2$), 22.58 (CH$_2$). MS (m/z): 425 (M^++1). Anal. calcd. for $C_{19}H_{13}ClN_6O_2S$: C, 53.71; H, 3.08; N, 19.78; found: C, 53.53; H, 3.20; N, 19.65.

3-(3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-6,7,8,9-tetrahydrobenzo[4,5]thieno[3,2-e][1,2,3]triazolo[1,5-a]pyrimidin-5(4H)-one 3g: Yield 74 %; yellow solid; m.p.: > 300ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (dd, J = 8.2, 5.7 Hz, 2H, H^{Ar}-2,6), 7.42 (t, J = 8.7 Hz, 2H, H^{Ar}-3,5), 2.95 (s, 2H, CH$_2$), 2.66 (s, 2H, CH$_2$), 1.80 (s, 4H, 2xCH$_2$). ^{13}C NMR (126 MHz, DMSO) δ 170.97 (C^{oxadiazole}-5), 167.24 (CO), 166.86 (C^{oxadiazole}-3), 164.29 (d, $^1J_{C\text{-}F}$ = 248.8 Hz, C^{Ar}-4), 152.88 (CTh-3), 145.10 (C^{Tr}-5), 130.13 (d, $^3J_{C\text{-}F}$ = 9.0 Hz, 2xCH^{Ar}-2,6), 130.12 (CTh-5), 125.73 (CTh-4), 123.94 (d, $^4J_{C\text{-}F}$ = 3.1 Hz, C^{Ar}-1), 116.74 (d, $^2J_{C\text{-}F}$ = 21.3 Hz, 2xCH^{Ar}-3,5), 116.66 (C^{Tr}-4), 110.98 (CTh-2), 26.25 (CH$_2$), 25.16 (CH$_2$), 23.21 (CH$_2$), 22.57 (CH$_2$). MS (m/z): 409 (M^++1). Anal. calcd. for $C_{19}H_{13}FN_6O_2S$: C, 55.88; H, 3.21; N, 20.58; found: C, 55.70; H, 3.28; N, 20.47.

3-(3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl)-6,7,8,9-tetrahydrobenzo[4,5]thieno[3,2-e][1,2,3]triazolo[1,5-a]pyrimidin-5(4H)-one 3h: Yield 72 %; yellow solid; m.p.: > 300ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (d, J = 8.2 Hz, 2H, H^{Ar}-3,5), 7.65 (d, J = 8.2 Hz, 2H, H^{Ar}-3,5), 2.95 (s, 2H, CH$_2$), 2.66 (s, 2H, CH$_2$), 1.86 – 1.72 (m, 4H, 2xCH$_2$). ^{13}C NMR (126 MHz, DMSO) δ 171.04 (C^{oxadiazole}-5), 167.24 (CO), 166.87 (C^{oxadiazole}-3), 152.88 (CTh-3), 145.09 (C^{Tr}-5), 136.40 (C^{Ar}-4), 130.11 (CTh-5), 129.76 (2xCH^{Ar}-3,5), 129.42 (2xCH^{Ar}-2,6), 126.23 (C^{Ar}-1), 125.78 (CTh-4), 116.64 (C^{Tr}-4), 111.01 (CTh-2), 26.25

(CH_2), 25.16 (CH_2), 23.21 (CH_2), 22.57 (CH_2). MS (m/z): 425 (M^++1). Anal. calcd. for $C_{19}H_{13}ClN_6O_2S$: C, 53.71; H, 3.08; N, 19.78; found: C, 53.69; H, 3.10; N, 19.79.

Conclusion

Thus, a new version of the domino reaction involving 2-(3-aryl-1,2,4-oxadiazol-5-yl)acetonitrile and 2-aminothiophene-3-carboxylates was studied. It was found that reaction occurred quickly at room temperature with the formation of new thieno[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine-5 (*4H*)-ones in high yields. The advantage of this method is that the reaction was carried out at room temperature and the products were formed in a single step with the high atom economy. The anticancer activity of these compounds was evaluated, however, the results demonstrated insufficient activity. Results of the current study can be used both for the synthesis of polycyclic thieno[2,3-d]pyrimidines and for the evaluation of their biological activity.

Acknowledgments

The authors thank the Ministry of Education and Science of Ukraine for financial support of this project (grant № 0121U107777).

References

[1] Ali, E. M., Abdel-Maksoud, M. S., Oh, C. H. (2019). Thieno[2,3-*d*]pyrimidine as a promising scaffold in medicinal chemistry: Recent advances. *Bioorg. Med. Chem., 27*(7), 1159–1194. https://doi.org/10.1016/j.bmc.2019.02.044

[2] Wilding, B., Klempier, N. (2017). Newest Developments in the Preparation of Thieno[2,3-*d*]pyrimidines. *Org. Prep. Proced. Int., 49*(3), 183–215. https://doi.org/10.1080/00304948.2017.1320513

[3] Abdelmoniem, A. M., Ghozlan, S. A., Abdelwahab, H. M., Abdelhamid, I. A. (2019). 2-Cyano-*N*-(thiophen-2-yl)acetamide in Heterocyclic Synthesis: Synthesis and Antibacterial Screening of Novel Pyrido[1,2-*a*]thieno[3,2-*e*]pyrimidine-2-carboxylate Moieties. *J. Heterocycl. Chem., 56*(9), 2637–2643. https://doi.org/10.1002/jhet.3676

[4] Mahmoud, M. R., Abu El-Azm, F. S., Ismail, M. F., Hekal, M. H., Ali, Y. M. (2018). Synthesis and antitumor evaluation of novel tetrahydrobenzo[4′,5′]thieno-[3′,2′:5,6] pyrimido[1,2-*b*]isoquinoline derivatives. *Synth. Commun., 48*(4), 428–438. https://doi.org/10.1080/00397911.2017.1406520

[5] Maloney, D. J., Waterson, A. G., Bantukallu, G. R., Brimacombe, K. R., Christov, P., Dang, V. Darley-Usmar, C. V., Hu, X., Jadhav, A., Jana, S., Kim, K. (2016). *WO Patent No. 2016109559 (A2).*

[6] Ivashchenko, A. A., Ivashchenko, A. V., Savchuk, N. F. (2009). *WO Patent No. 2009093934 (A2).*

[7] Kim, J., Kwon, J., Lee, D., Jo, S., Park, D. S., Choi, J., Park, E., Hwang, J. Y., Ko, Y., Choi, I., Ju, M. K., Ahn, J. Y., Kim, J,, Han, S.-J., Kim, T.-H., Cechetto, J., Nam, J., Ahn, S., Sommer, P., Liuzzi, M., No, Z., Lee, J. (2013). Synthesis and biological evaluation of triazolothienopyrimidine derivatives as novel HIV-1 replication inhibitors. *Bioorg. Med. Chem. Lett., 23*(1), 153–157. https://doi.org/10.1016/j.bmcl.2012.10.134

[8] Shyyka, O., Pokhodylo, N., Finiuk, N., Matiychuk, V., Stoika, R., Obushak, M. (2018). Anticancer Activity Evaluation of New Thieno[2,3-*d*]pyrimidin-4(*3H*)-ones and Thieno[3,2-*d*]pyrimidin-4(*3H*)-one Derivatives. *Sci. Pharm., 86*(3), 28. https://doi.org/10.3390/scipharm86030028

[9] Shyyka, O. Y., Pokhodylo, N. T., Palchykov, V. A., Finiuk, N. S., Stoika, R. S., Obushak, M. D. (2020). Cage-like amines in the green protocol of transannular thieno[2,3-*d*]pyrimidinone formation as promising anticancer agents. *Chem. Heterocycl. Compd., 56*, 793–799. https://doi.org/10.1007/s10593-020-02732-2

[10] Shyyka, O. Ya., Pokhodylo, N. T., Finiuk, N. S. (2019). Anticancer activity evaluation of thieno[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidines and thieno[2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine derivatives. *Biopolym. Cell, 35*(2), 321–330. http://dx.doi.org/10.7124/bc.000A0F

[11] Renyu, Q., Yuchao, L., Kandegama, W. M., Qiong, C., Guangfu, Y. (2018). Recent applications of triazolopyrimidine-based bioactive compounds in medicinal and agrochemical chemistry. *Mini Rev. Med. Chem., 18*(9), 781–793. https://doi.org/10.2174/1389557517666171101112850

[12] Singh, P. K., Choudhary, S., Kashyap, A., Verma, H., Kapil, S., Kumar, M., Arora, M., Silakari, O. (2019). An exhaustive compilation on chemistry of triazolopyrimidine: A journey through decades. *Bioorg. Chem., 88*, 102919. https://doi.org/10.1016/j.bioorg.2019.102919

[13] Brand, S., Ko, E. J., Viayna, E., Thompson, S., Spinks, D., Thomas, M., Sandberg, L., Francisco, A. F., Jayawardhana, S., Smith, V. C., Jansen, C., De Rycker, M., Thomas, J., MacLean, L., Osuna-Cabello, M., Riley, J., Scullion, P., Stojanovski, L., Simeons, F. R. C., Epemolu, O., Shishikura, Y., Crouch, S. D., Bakshi, T. S., Nixon, C. J., Reid, I. H., Hill, A. P., Underwood, T. Z., Hindley, S. J., Robinson, S. A., Kelly, J. M., Fiandor, J. M., Wyatt, P. G., Marco, M., Miles, T. J., Read, K. D., Gilbert, I. H. (2017). Discovery and Optimization of 5-Amino-1,2,3-triazole-4-carboxamide Series against Trypanosoma cruzi. *J. Med. Chem., 60*(17), 7284–7299. https://doi.org/10.1021/acs.jmedchem.7b00463

[14] Yang, Z., Shen, M., Tang, M., Zhang, W., Cui, X., Zhang, Z., Pei, H., Li, Y., Hu, M., Bai, P., Chen, L. (2019). Discovery of 1,2,4-oxadiazole-Containing hydroxamic acid derivatives as histone deacetylase inhibitors potential application in cancer therapy. *Eur. J. Med. Chem., 178*, 116–130. https://doi.org/10.1016/j.ejmech.2019.05.089

[15] Caneschi, W., Enes, K. B., de Mendonça, C. C., de Souza Fernandes, F., Miguel, F. B., da Silva Martins, J., Le Hyaric, M.; Pinho, R. R.; Duarte, L. M.; Leal de Oliveira, M. A.; Dos Santos, H. F.; Paz Lopes, M. T.; Dittz, D.; Silva, H.; Costa Couri, M. R. (2019). Synthesis and anticancer evaluation of new lipophilic 1,2,4 and 1,3,4-oxadiazoles. *Eur. J. Med. Chem., 165*, 18–30. https://doi.org/10.1016/j.ejmech.2019.01.001

[16] Mohammadi-Khanaposhtani, M., Ahangar, N., Sobhani, S., Masihi, P. H., Shakiba, A., Saeedi, M., Akbarzadeh, T.

(2019). Design, synthesis, in vivo, and in silico evaluation of new coumarin-1, 2, 4-oxadiazole hybrids as anticonvulsant agents. *Bioorg. Chem., 89*, 102989. https://doi.org/10.1016/j.bioorg.2019.102989

[17] De, S. S., Khambete, M. P., Degani, M. S. (2019). Oxadiazole scaffolds in anti-tuberculosis drug discovery. *Bioorganic Med. Chem. Lett., 29*(16), 1999–2007. https://doi.org/10.1016/j.bmcl.2019.06.054

[18] Pokhodylo, N. T. (2015). Multicomponent and domino reactions leading to 1,2,3-triazoles. Chemistry of 1,2,3-triazoles. In W. Dehaen, V. A. Bakulev (Eds.), *Topics in Heterocyclic Chemistry,* (vol. 40, Chemistry of 1,2,3-triazoles, pp. 269-324). Switzerland: Springer International Publishing. https://doi.org/10.1007/7081_2014_122

[19] Westerlund, C. (1980). The synthetic utility of heteroaromatic azido compounds. Part VI. Preparation of some 1H-1,2,3-triazolo[1,5-*a*]thieno[3,2-*d*] pyrimidines. *J. Heterocycl. Chem., 17*(8), 1765–1769. https://doi.org/10.1002/jhet.5570170826

[20] Lauria, A., Patella, C., Abbate, I., Martorana, A., Almerico, A. M. (2013). An unexpected Dimroth rearrangement leading to annelated thieno[3,2-*d*][1,2,3]triazolo[1,5-*a*]pyrimidines with potent antitumor activity. *Eur. J. Med. Chem., 65*, 381–388. https://doi.org/10.1016/j.ejmech.2013.05.012

[21] Ivachtchenko, A. V., Golovina, E. S., Kadieva, M. G., Koryakova, A. G., Kovalenko, S. M., Mitkin, O. D., Okun, I. M., I. Ravnyeyko, M., Tkachenko S. E., Zaremba, O. V. (2010). Synthesis and biological study of 3-(phenylsulfonyl)thieno[2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidines as potent and selective serotonin 5-HT6 receptor antagonists. *Bioorg. Med. Chem., 18*(14), 5282–5290. https://doi.org/10.1016/j.bmc.2010.05.051

[22] Anderson, M. O., Zhang, J., Liu, Y., Yao, C., Phuan, P. W., Verkman, A. S. (2012). Nanomolar potency and metabolically stable inhibitors of kidney urea transporter UT-B. *J. Med. Chem., 55*(12), 5942–5950. https://doi.org/10.1021/jm300491y

[23] Pokhodylo, N. T., Matiychuk, V. S., Obushak, N. D. (2009). Synthesis of a new heterocyclic system–pyrido[3',2':4,5]thieno-[2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine. *Chem. Heterocycl. Compd., 45*(7), 881–883. https://doi.org/10.1007/s10593-009-0338-z

[24] Lauria, A., Diana, P., Barraja, P., Almerico, A. M., Cirrincione, G., Dattolo, G. (2000). Pyrrolo[3,4-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine and pyrrolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine. New tricyclic ring systems of biological interest. *J. Heterocycl. Chem., 37*(4), 747–750. https://doi.org/10.1002/jhet.5570370413

[25] Lauria, A., Diana, P., Barraja, P., Montalbano, A., Cirrincione, G., Dattolo, G., Almerico, A. M. (2002). New tricyclic systems of biological interest. Annelated 1,2,3-triazolo[1,5-*a*]pyrimidines through domino reaction of 3-azidopyrroles and methylene active nitriles. *Tetrahedron, 58*(48), 9723–9727. https://doi.org/10.1016/S0040-4020(02)01245-0

[26] Lauria, A., Patella, C., Diana, P., Barraja, P., Montalbano, A., Cirrincione, G., Dattolo, G., Almerico A. M. (2003). A New Tetracyclic Ring System of Biological Interest. Indolo[3,2-*e*]-[1,2,3]triazolo[1,5-*a*]pyrimidines through Domino Reactions of 2-Azidoindole. *Heterocycles, 60*(12), 2669–2675. https://doi.org/10.3987/COM-03-9877

[27] Lauria, A., Patella, C., Diana, P., Barraja, P., Montalbano, A., Cirrincione, G., Dattolo G., Almerico, A. M. (2006). A synthetic approach to new polycyclic ring system of biological interest through domino reaction: indolo[2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine. *Tetrahedron Lett., 47*(13), 2187–2190. https://doi.org/10.1016/j.tetlet.2006.01.112

[28] Lauria, A., Patella, C., Dattolo, G., Almerico, A. M. (2008). Design and Synthesis of 4-Substituted Indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine Derivatives with Antitumor Activity. *J. Med. Chem., 51*(7), 2037–2046. https://doi.org/10.1021/jm700964u

[29] Khan, M. A., Freitas, A. C. C. (1980). Fused pyrazolopyrimidines. I. Pyrazolo[4,3-*e*]-v-triazolo[1,5-*a*]pyrimidine. A new heterocyclic system. *J. Heterocycl. Chem., 17*(7), 1603–1604. https://doi.org/10.1002/jhet.5570170751

[30] Lauria, A., Abbate, I., Patella, C., Gambino, N., Silvestri, A., Barone, G., Almerico, A. M. (2008). Pyrazolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine: a new ring system through Dimroth rearrangement. *Tetrahedron Lett., 49*(35), 5125–5128. https://doi.org/10.1016/j.tetlet.2008.06.104

[31] Paula Freitas, A., Fernanda, M., Proença, J. R. P., Booth, B. L. (1995). Synthesis of 5-azido-4-cyanoimidazole and its reaction with active methylene compounds. *J. Heterocycl. Chem., 32*(2), 457–462. https://doi.org/10.1002/jhet.5570320212

[32] L'abbé, G., Godts, F., Toppet, S. (1985a). Synthesis of 5-Diazomethyl Substituted V-Triazolo[4,5-*d*]Pyrimidines. *Bull. Soc. Chim. Belg., 94*(7), 441–447. https://doi.org/10.1002/bscb.19850940702

[33] L'abbé, G., Godts, F., Toppet, S. (1985b). Diazoimine–triazole equilibrium in fused 1,2,3-triazolo[1,5-*a*]pyrimidines. *J. Chem. Soc., Chem. commun.,* (9), 589–590. https://doi.org/10.1039/C39850000589

[34] L'abbé, G., Godts, F., Toppet, S., Meervelt, L. V., King, G. S. (1987). Synthesis and Crystal Structure of *Bis*[1,2,3]Triazolo[1,5-*a*:4′,5′-*d*] Pyrimidin-9-Ones. *Bull. Soc. Chim. Belg., 96*(8), 587–593. https://doi.org/10.1002/bscb.19870960805

[35] Pokhodylo, N. T., Shyyka, O. Y., Obushak, M. D. (2014). Facile and efficient one-pot procedure for thieno[23-*e*][1,2,3]triazolo[1,5-*a*]pyrimidines preparation. *Synth. Commun., 44*(7), 1002–1006. https://doi.org/10.1080/00397911.2013.840729

[36] Pokhodylo, N. T., Shyyka, O. Y., Savka, R. D., Obushak, M. D. (2010). Novel selected tandem transformations of the amino and carbonyl/nitrile groups in the Gewald thiophenes. *Phosphorus, Sulfur, and Silicon, 185*(10), 2092–2100. https://doi.org/10.1080/10426500903496739

[37] Pokhodylo, N. T., Shyyka, O. Y., Tupychak, M. A., Obushak, M. D. (2018). Selectivity in domino reaction of ortho-carbonyl azides with malononitrile dimer leading to [1,2,3]triazolo[1,5-*a*]pyrimidines. *Chem. Heterocycl. Compd., 54,* 209–212. https://doi.org/10.1007/s10593-018-2256-4

[38] Pokhodylo, N. T., Shyyka, O. Y. (2017). New cascade reaction of azides with malononitrile dimer to polyfunctional [1,2,3]triazolo[4,5-b]pyridine. *Synth. Commun., 47*(11), 1096–1101. https://doi.org/10.1080/00397911.2017.1313427

[39] Tupychak, M. A., Shyyka, O. Y., Pokhodylo, N. T., Obushak, M. D. (2020). Nitrileimines as an alternative to azides in base-mediated click [3+2]cycloaddition with methylene active nitriles. *RSC Adv., 10*(23), 13696–13699. https://doi.org/10.1039/D0RA01417F

[40] Shoemaker, R. H. (2006). The NCI60 human tumour cell line anticancer drug screen. *Nat. Rev. Cancer., 6*(10), 813–823. https://doi.org/10.1038/nrc1951

[41] Grever, M. R., Schepartz, S. A., Chabner, B. A. (1992). The National Cancer Institute: cancer drug discovery and development program. *Semin. Oncol.. 19*(6), 622–638.

[42] Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paull, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-Goodrich, M., Campbell, H., Mayo, J., Boyd M. (1991). Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Natl. Cancer Inst.,* 83(11), 757–766, https://doi.org/10.1093/jnci/83.11.757

[43] Boyd, M. R., Paull, K. D. (1995). Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. *Drug Dev. Res., 34*(2), 91–109. https://doi.org/10.1002/ddr.430340203