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Synthesis and evaluation of cytotoxic and antimicrobial activity of some 3-aryl-6-phenyl-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines

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A B S T R A C T

3-aryl-6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines were obtained with good yields (81-95%) via the heterocyclization reaction of 1-phenyl-2-((5-aryl-1,3,4-oxadiazole-2-yl)thio)ethane-1-ones in acetic acid. The physicochemical characteristics of the synthesized compounds were established, the structures were confirmed by the data of IR, ^{1}H and ^{13}C NMR spectra, as well as the results of X-ray diffraction analysis. The cytotoxic, antibacterial and antifungal properties of these compounds were evaluated. *In vitro* screening results showed that compounds **8**, **9** and **12** significantly inhibit (54-65%) the growth of *HeLa*, *HBL-100* and *CCRF-CEM* cancer cell lines. It was found that the cytotoxicity of the synthesized compounds increases in the series of oxadiazoltiones (**1**-**4**) - *S*-derivatives (**5**-**8**) - triazolothiadiazines (**9**-**12**). Compounds **5-16** do not exhibit antimicrobial properties.

1. Introduction

Currently, one of the most widespread and widely studied class of organic compounds are nitrogen-, oxygen- and sulfur-containing five-membered heterocycles - 1,3,4-oxadiazoltiones, 1,3,4-thiadiazoltiones and 1,2,4-triazoltiones. Recently, numerous derivatives of 1,3,4-oxa(tia)diazoles and 1,2,4-triazoles have been reported to have various types of biological activity, such as antibacterial, antitumor, antiviral, antihypertensive, analgesic, anti-inflammatory, etc.[1-10] Along with their great practical significance 5-substituted-1,3,4-oxadiazole-2-thiones are particularly attractive to most researchers due to the presence of a thioamide group - NH-C=S. Depending on the nature of the electrophilic reacting agent and the experimental conditions, it is possible to obtain derivatives at two nucleophilic reaction centers of this group - both at the exocyclic sulfur atom and at the endocyclic nitrogen atom located in the oxadiazole heterocycle. This is of great interest from the point of view of the synthesis of biologically active derivatives of 1,3,4-oxadiazole-2-thiones.[11-14]

This work is devoted to the synthesis of a number of 3-aryl-6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines by the heterocyclization of 1-phenyl-2-((5-aryl-1,3,4-oxadiazole-2-yl)thio)ethane-1-ones. The synthetic approach we implemented can be considered as an alternative route for the synthesis of such heterocyclic compounds. In many studies, such compounds are prepared from the corresponding 4-amino-5-aryl(heteryl)-3H-1,2,4-triazole-3-thiones.[15-25] Also a comparative study of the cytotoxic and antimicrobial activity of the starting oxadiazole-2-thiones, synthesized S-derivatives and triazolo-thiadiazines is performed.

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2. Results and Discussion

Continuing of our research on the synthesis of new derivatives of 5-substituted-1,3,4-oxadiazoltiones[26-28] in the present work we obtained 3-aryl-6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **9-12** and evaluated their cytotoxic and antimicrobial activity. 1-Phenyl-2-((5-aryl-1,3,4-oxadiazole-2-yl)thio)ethane-1-ones **5-8** were used as starting substances for the synthesis of the corresponding thiadiazines **9-12** which were obtained under selective conditions leading exclusively to *S*-alkyl derivatives (the ratio of oxadiazolthiones (**1-4**) and 2-bromoacetophenone 1:1, boiling in anhydrous acetone in the presence of K_2CO_3):

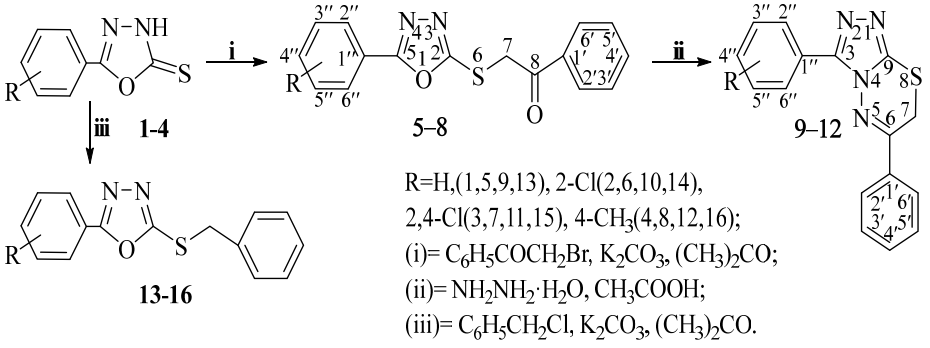

Fig. 1. The scheme for the synthesis of compounds **5-16**.

The structures of compounds **5-8** are confirmed by the data of spectral methods: in the IR spectra there are absorption bands of the C=O group in the range of 1675-1680 cm^{-1}, and in the ^1H NMR spectra, signals of protons of the S-CH_2 group are observed in the form of a singlet at 4.97-5.07 ppm. [29-30] There are signals of all protons of both aromatic rings of compounds **5-8** in the range of 7.25-8.07 ppm. In ^{13}C NMR spectra **5-8** the characteristic signals of carbon atoms of the methylene group S-CH_2 (C-7) and C=O group (C-8) are observed at 41.15-41.66 ppm and 192.13-192.40 ppm respectively.

As it is known, the keto group in the composition of compounds can react with nucleophilic reagents, leading to various derivatives, including fragments of the 1,2,4-triazole cycle. Based on this, the next stage of our study was the cyclization reactions of synthesized thioketones **5-8** with hydrazine hydrate. The interaction was carried out by boiling in concentrated acetic acid, as a result, products **9-12** were obtained in the form of crystals in good yields (81-95%).

In the ^1H NMR spectrum of compounds **9-12** singlet signals of methylene protons of the S-CH_2 group of thioketones **5-8** at 4.97-5.07 ppm are shifted into a stronger field of 3.99-4.33 ppm. [21] At the same time, there is a disappearance in the IR spectra of **9-12** signals of the C=O group within 1675-1680 cm^{-1}, the C–O-C group at 1175-1189 cm^{-1} and the appearance of absorption at 1595-1612 cm^{-1} for the C=N group[22,23,31-34] at the carbon atom C-6. The absence of a signal in the ^{13}C NMR spectra of compounds of **9-12** carbon atom of the C=O group at 192.13-192.40 ppm and the shifting of the signal of the methylene group S-CH_2 in a strong field of 23.15-23.67 ppm together with the data of ^1H NMR and IR spectra shows that synthesized bicyclic heterocyclic compounds - 3-aryl-6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **9-12**.

To reliably establish the structure of 1-phenyl-2-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)ethan-1-one (**5**) and 3,6-diphenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (**9**) the X-ray diffraction analysis was performed. The molecular structure of the compound **5** is shown in **Fig. 2**.

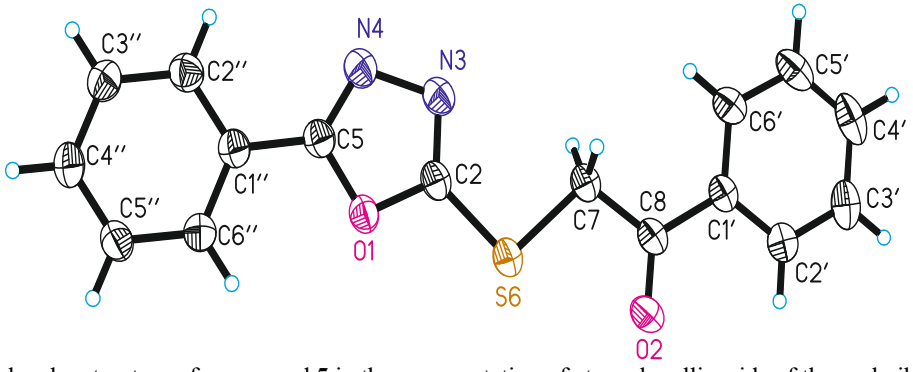

Fig. 2. The molecular structure of compound **5** in the representation of atoms by ellipsoids of thermal vibrations with a 30% probability

As can be seen from Figure 1, the molecule consists of three aromatic fragments. The angles between the oxadiazole and the two benzene rings are 5.1(2)° and 5.5(2)°. In the crystal structure **5**, weak intermolecular hydrogen bonds of the C–H...O type are observed, which link the molecules along the *c*-axis direction. The parameters of this hydrogen bond are as follows: distances C5′…O2 3.371(4), H…O2 2.49 Å, angle C5′–H5′B…O2 158º (*x, 1/2-y, -1/2+z*).

The obtained X-ray crystallography data of compound **9** confirm its structure as 3,6-diphenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine and its crystallographic parameters are the same as previously published ones.[35]

In vitro cytotoxic and antimicrobial activity of synthesized compounds

Triazolothiadiazines and their various derivatives consisting pharmacologically active fragments open up wide opportunities for the creation of promising drugs. The literature describes many examples of the biological activity of compounds containing a 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine ring. They have antimicrobial, antitumor, antiviral, anti-inflammatory, antioxidant, antituberculosis and other types of biological activity.[18-20,32-34,36-37]

A number of studies have shown that various derivatives of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines exhibit anticancer properties. Thus, Aytac et al.[38] developed and synthesized a series of 1,2,4-triazolo[3,4-*b*][1,3,4] thiadiazines, which were studied *in vitro* against liver (*Huh7*), breast (*MCF-7*) and colon cancer cell lines (*HCT116*). According to the results, all triazolothiadiazine derivatives have a significant cytotoxic potential (IC_{50} = 1.1 - 18.8 μM). Xu et al.[16] synthesized 3,6-diaryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines as antiproliferative agents against gastric adenocarcinoma cancer cells (*SGC-7901*), lung adenocarcinoma (*A549*) and fibrosarcoma (*HT-1080*). Some compounds of this series showed excellent activity with values of IC_{50}=0.011 - 0.079 μM. At the same time, the researchers indicated the importance of substituents at position 3 of the triazothiadiazine framework on the activity exhibited. Khan et al.[39] screened the antitumor activity of several 6-aryl-3-(pyridin-4-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazines on kidney fibroblast (*BHK-21*) and lung carcinoma (*H157*) cell lines at four different concentrations. One of the compounds tested showed the highest efficacy with 78.6% inhibition (100 μM) against *H157* cells, which was better than the vincristine standard (74.5%). The SAR studies have revealed the importance of aryl substituents at position - 6 of triazolothiadiazine for antitumor activity. Zhang et al.[17] identified novel 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines that exhibit strong antiproliferative activity against *PC-3* cell lines (prostate cancer cells) and *A549* (alveolar epithelial cells). Farghaly et al.[18] conducted an antitumor evaluation of 3,6,7-trisubstituted-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines and identified compounds among them with potential activity against cellular *HEPG-2* and *HCT* lines with IC_{50} values of 8.63 and 8.33 μg mL^{-1}, respectively.

Based on the above described in this work we have studied the cytotoxicity of the synthesized 1-phenyl-2-((5-aryl-1,3,4-oxadiazol-2-yl)thio)ethan-1-ones **5-8**, 3-aryl- 6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **9-12** and the starting 5-aryl-1,3,4-oxadiazol-2-thiones **1-4**.

Table 1. Results of cytotoxicity of the compounds **1-16** on cancer cell lines *HEp-2*, *HeLa*, *HBL-100* and *CCRF-CEM*.

Compounds, 100 μM	Cell line Inhibition of cell growth, %			
	HEp-2	***HeLa***	***HBL-100***	***CCRF-CEM***
1	8.9±0.6	23.9±1.8	3.9±0.6	29.5±1.2
2	16.0±0.4	45.0±2.2	16.5±0.8	27.9±1.2
3	4.1±0.2	27.4±0.9	8.3±0.7	52.6±1.7
4	proliferation 8%	proliferation 8,8%	proliferation 7,5%	proliferation 17,5%
5	0.0±0.0	0.7±1.1	34.9±1.8	35.5±1.6
6	12.4±2.1	0.0±0.0	0.0±0.0	37.1±1.6
7	19.5±6.2	23.7±1.9	17.7±1.2	13.0±1.1
8	16.7±2.4	54.5±6.4	42.4±2.2	35.2±1.8
9	18.7±0.0	37.8±7.1	56.0±2.8	65.1±2.1
10	28.8±1.5	43.2±1.9	3.6±0.5	16.0±0.9
11	44.5±10.0	12.0±1.6	30.1±1.4	44.6±1.5
12	28.8±1.8	57.6±2.5	26.4±1.6	54.0±2.1
13	14.7±1.1	0.4±0.0	0.0±0.0	0.0±0.0
14	6.9±0.1	17.0±1.1	0.0±0.0	5.7±0.8
15	18.1±1.2	20.0±2.1	1.7±0.1	24.3±0.7
16	19.2±0.9	35.2±1.3	0.0±0.0	23.5±2.1
Cisplatin	79.1±2.3	100.0±1.8	87.9±2.5	63.1±1.6

As can be seen from Table 1, the molecule of 5-phenyl-1,3,4-oxadiazol-2-thione (**1**) itself did not exhibit pronounced cytotoxicity towards the presented cell lines. The introduction of a Cl atom in the ortho position of the phenyl ring (compound **2**) promoted the appearance of weak activity of the molecule in a cervical carcinoma (*HeLa*) cell line. With the introduction of a second chlorine atom (compound **3**), cytotoxicity increased against T-lymphoblastic leukemia cells - up

to 52.6% inhibition of cell growth at 100 μM. In contrast to these data, the presence of a methyl group in the para-position of the phenyl ring (compound **4**) contributed to the opposite effect and proliferation of cells of all studied lines. Compounds **5**-**8**, obtained by introducing a phenacyl (-$CH_2C(O)C_6H_5$) group into the sulfur atom of the 1,3,4-oxadiazole heterocycle, showed an ambiguous effect. Thus, an increase in cytotoxicity was observed in 1-phenyl-2-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)ethan-1-one (**5**), which does not have substituents in the phenyl ring on *HBL*- cells 100 and *CCRF-CEM* (34.9 and 35.5%, respectively) in contrast to the original compound **1**. However, the activity of compounds **6** and **7** with Cl atoms in the phenyl ring was low or completely absent. It is interesting to note that the introduction of the phenacyl group contributed to the disappearance of the proliferative properties observed in the starting compound **4** in all studied cells. This led to the manifestation of moderate activity of substance **8**, which has a methyl group in the phenyl ring, on *HEp-2* and *CCRF-CEM* cells, and on the *HeLa* and *HBL-100* lines - even more pronounced cytotoxicity (54.5 and 42.4% of cell death, respectively).

Replacement of the phenacyl (-$CH_2C(O)C_6H_5$) group in the S atom of 5-aryl-1,3,4-oxadiazol-2-thiones **1**-**4** with a benzyl (-CH_2C6H_5) substituent in compounds 13-16 does not effect on cytotoxicity, both in a molecule without Cl atoms (**13**) and in their presence (**14**,**15**). The presence of a methyl radical together with a benzyl group, as in the case of **8**, led to the loss of proliferative activity of the compound (**16**) and the manifestation of slight cytotoxicity on *HeLa* and *CCRF-CEM* cells.

Further modification of 5-aryl-1,3,4-oxadiazol-2-thiones with the introduction of the -[1,2,4]triazolo[3,4-*b*]-1,3,4-thiadiazine group of the molecule led to a significant increase in cytotoxicity. . At the same time, compound **9** showed pronounced inhibitory properties on the *HBL-100* and *CCRF-CEM* cell lines - 56 and 65.1% cell inhibition, respectively, and moderate (37.8%) on the *HeLa* line. Substance **12** with a methyl group in the para-position of the phenyl ring also showed a pronounced effect on cells of cervical carcinoma *HeLa* (57.6%) and T-lymphoblastic leukemia *CCRF-CEM* (54.0%). The presence of one chlorine atom in compound **10** led to a decrease in cytotoxicity towards most of the tested (*HEp-2*, *HBL-100*, *CCRF-CEM*) cells, while compound **11** with two chlorine atoms had higher activity on *HEp-2* cells (44.5 %) and *CCRF-CEM* (44.6%).

A comparison of data on the cytotoxicity of the studied compounds showed that their activity increases in the following sequence - initial oxadiazolethiones (**1**-**4**) -- *S*-derivatives (**5**-**8**) -- triazothiadiazines (**9**-**12**).

Previously, we studied the antimicrobial properties of the original oxadiazolethiones **1**-**4**, which exhibit remarkable activity against gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* (diameters of inhibition zone 7-17 mm).[28] *In vitro* evaluation of antibacterial and antifungal activity of compounds 5-16 did not present any antimicrobial properties.

3. Conclusions

In this work the synthesis of aryl-substituted 7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines was described. The synthetic approach to obtaining these derivatives differs from the methods described in the literature, where similar compounds were obtained from 4-amino-5-aryl(heteryl)-3H-1,2,4-triazole-3-thiones. *In vitro* screening showed that compounds 8, 9 and 12 exhibited significant cytotoxicity in *HeLa, HBL-100 and CCRF-CEM* cell lines. It has also been established that for the cytotoxic effect to occur, the presence of a triazothiadiazine heterocycle in aryl-substituted 7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines is necessary, which corresponds to literature data.[16-18,22,32] Thus, 3-aryl-substituted-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines may be promising compounds for the search of cytotoxic agents.

Acknowledgement

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4. Experimental

4.1. Instrumentation

IR spectra were recorded on an FT-IR/NIR Spectrum 3 spectrometer (Perkin Elmer, Switzerland) using an ATR system. ^{1}H and ^{13}C NMR spectra were recorded on a JNM-ECZ600R spectrometer (JEOL, Japan) at an operating frequency of 600 MHz for ^{1}H in DMSO-d_6+CCl_4 solutions. TMS (0 ppm) was used as an internal standard in ^{1}H NMR spectra. In the ^{13}C NMR spectra, the chemical shift of the solvent (DMSO-d_6, 39.52 ppm relative to TMS) was used as an internal standard. Mass spectra were run using Agilent Technologies 6520 Accurate-Mass Q-TOF LC/MS. TLC was carried out on ALUGRAM® SIL G/UV254 plates ($CHCl_3$-EtOH, 24:1), development under UV light. The melting point was determined using a “BOETIUS” device.

4.2. General procedure

Compounds **1**-**4** and **13**-**16** were synthesized according to the procedure.[40,41]

*4.2.1. General procedure for the synthesis of 1-phenyl-2-((5-aryl-1,3,4-oxadiazol-2-yl)thio)ethan-1-ones **5-8**.*

0.05 mol of 5-aryl-1,3,4-oxadiazol-2-thiones **1-4**, bromoacetophenone, K_2CO_3 and 15 ml of dry acetone were placed in a round-bottom flask equipped with an effective reflux condenser. The reactions were carried out by boiling the mixture for 4-5 hours (the progress of the reactions was monitored by TLC). Then the solvent was removed from the mixture, the residue was placed in a funnel with a glass filter and washed with NaOH solution (2%) to remove unreacted starting thione, then washed with water until neutral. After drying in air, the target reaction products **5-8** were obtained.

*4.2.2. 1-Phenyl-2-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)ethan-1-one (**5**).* Yield 91%, white solid, m.p.163-164°C, R_f=0,68. ^{1}H NMR (600 MHz, DMSO-d_6+CCl_4, ppm) δ: 5.06 (2H, s, S-C<u>H</u>$_2$), 7.49-7.55 (5H, m, Ar-H- 4″,3′,5′,3″,5″), 7.64 (1H, t, J=7.4, Ar-H-4′), 7.91-7.93 (2H, m, Ar-H-2′,6′), 8.03-8.05 (2H, m, Ar-H-2″,6″). ^{13}C NMR (150 MHz, DMSO-d_6+CCl_4, ppm) δ: 41.15 (C-7), 123.73 (C-1″), 126.77 (C-2″,6″), 128.93 (C-3′,5′), 129.14 (C-2′,6′), 129.53 (C-3″,5″), 132.06 (C-4″), 134.14 (C-4′), 135.55 (1′), 163.72 (C-2), 165.52 (C-5), 192.40 (C-8). IR, ν, cm^{-1}: 688 (C-S), 1186 (C-O-C), 1675 (C=O). ESI-MS: *m/z* calculated 296.06, found 297.08 [M+H]+.

*4.2.3. 1-Phenyl-2-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)ethan-1-one (**6**).* Yield 87%, white solid, m.p.121-122°C, R_f=0,53. ^{1}H NMR (600 MHz, DMSO-d_6+CCl_4, ppm) δ: 5.07 (2H, s, S-C<u>H</u>$_2$), 7.45 (1H, t, J=7.4, Ar-H-4″), 7.50-7.57 (4H, m, Ar-H-5″,3′,5′,4′), 7.62-7.66 (1H, m, Ar-H-3″), 7.90 (1H, d, J=7.8, Ar-H-6″) 8.03 (2H, m, Ar-H-2′, 6′). ^{13}C NMR (150 MHz, DMSO-d_6+CCl_4, ppm) δ: 41.19 (C-7), 122.88 (C-1″), 127.81 (C-5″), 128.91 (C-3′, 5′), 129.14 (C-6′), 129.16 (C-2′), 131.36 (C-6″), 131.49 (C-3″), 132.53 (C-4″), 133.13 (C-4′), 134.16 (C-2″), 135.55 (C-1′), 163.69 (C-2), 164.40 (C-5), 192.18 (C-8). IR, ν, cm^{-1}: 687 (C-S), 1178 (C-O-C), 1677 (C=O). ESI-MS: *m/z* calculated 330.02, found 331.03 [M+H]+.

*4.2.4. 1-Phenyl-2-((5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl)thio)ethan-1-one (**7**).* Yield 94%, pale yellow solid, m.p. 167-168°C, R_f=0,49. ^{1}H NMR (600 MHz, DMSO-d_6+CCl_4, ppm) δ: 5.07 (2H, s, S-C<u>H</u>$_2$), 7.49-7.53 (3H, m, Ar-H- 5″,3′,5′), 7.62-7.65 (2H, m, Ar-H-3″,4′), 7.93 (1H, d, J=8.5, Ar-H-6″), 8.02-8.04 (2H, m, Ar-H-2′, 6′). ^{13}C NMR (150 MHz, DMSO-d_6+CCl_4, ppm) δ: 41.20 (C-7), 121.74 (C-1″), 128.31 (C-5″), 128.91 (C-3′, 5′), 129.13 (C-2′,6′), 131.12 (C-6″), 132.49 (C-3″), 133.43 (C-4′), 134.13 (C-2″), 135.53 (C-1′), 137.73 (C-4″), 162.96 (C-2), 164.64 (C-5), 192.13 (C-8). IR, ν, cm^{-1}: 688 (C-S), 1189 (C-O-C), 1680 (C=O). ESI-MS: *m/z* calculated 363.98, found 364.99 [M+H]+.

*4.2.5. 1-Phenyl-2-((5-(p-tolyl)-1,3,4-oxadiazol-2-yl)thio)ethan-1-one (**8**).* Yield 86%, white solid, m.p. 152-153°C, R_f=0,57. ^{1}H NMR (600 MHz, DMSO-d_6+CCl_4, ppm) δ: 2.41 (3H, s, -C<u>H</u>$_3$) 4.97 (2H, s, S-C<u>H</u>$_2$), 7.28 (2H, dd, J=8.7, J=1.0, Ar-H-3″,5″), 7.49-7.54 (2H, m, Ar-H-3′,5′), 7.63 (1H, t, J=7.4, Ar-H-4′), 7.87 (2H, d, J=8.2, Ar-H-2″,6″), 8.03-8.06 (2H, m, Ar-H-2′,6′). ^{13}C NMR (150 MHz, DMSO-d_6+CCl_4, ppm) δ: 21.75 (<u>C</u>H$_3$-), 41.66 (C-7), 120.81 (C-1″), 126.78 (C-2″,6″), 128.66 (C-3′, 5′), 129.07 (C-2′,6′), 129.86 (C-3″,5″), 134.34 (C-4′), 135.02 (C-1′), 142.41 (C-4″), 163.42 (C-2), 166.27 (C-5), 192.32 (C-8). IR, ν, cm^{-1}: 684 (C-S), 1176 (C-O-C), 1679 (C=O). ESI-MS: *m/z* calculated 310.08, found 311.09 [M+H]+.

*4.2.6. Synthesis 3-aryl-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**9-12**).*

To a mixture of 0.026 mol 1-phenyl-2-((5-aryl-1,3,4-oxadiazol-2-yl)thio)ethan-1-one **5-8** in 6 ml acetic acid the 0.052 mol hydrazine hydrate (98%) was added. In this case, a slight release of white smoke occurs, and after 15-20 minutes, slow boiling began (7 h). After cooling to room temperature, the transparent, pale yellow reaction mixture was poured into crushed ice, the precipitate that formed was filtered off, and washed with water. The resulting substances (**9-12**) in the form of powders were recrystallized from ethanol.

*4.2.7. 3,6-Diphenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**9**).* Yield 95%, pale brown crystals, m.p. 216-217°C, R_f=0,41. ^{1}H NMR (600 MHz, DMSO-d_6+CCl_4, ppm) δ: 4.31 (2H, s, S-C<u>H</u>$_2$), 7.44-7.54 (6H, m, ArH-3′,5′,3″,5″,4′,4″), 7.97-8.01 (4H, m, ArH-2′,6′,2″,6″). ^{13}C NMR (150 MHz, DMSO-d_6+CCl_4, ppm) δ: 23.15 (C-7), 126.74 (C-1″), 127.98 (C-2″,6″), 128.31 (C-3′, 5′), 128.77 (C-3″,5″), 129.25 (C-2′,6′), 130.17 (C-1′), 131.93 (C-4′), 134.09 (C-4″), 142.58 (C-6), 152.04 (C-9), 155.46 (C-3). IR, ν, cm^{-1}: 690 (C-S), 1596 (C=N). ESI-MS: *m/z* calculated 292.08, found 293.08 [M+H]+.

*4.2.8. 3-(2-Chlorophenyl)-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**10**).* Yield 83%, small needle-shaped beige crystals, m.p. 177-178°C, R_f=0,48. ^{1}H NMR (600 MHz, DMSO-d_6+CCl_4, ppm) δ: 4.33 (2H, s, S-C<u>H</u>$_2$), 7.42-7.50 (4H, m, ArH-3′, 5′, 5″, 4″), 7.54 (1H, t, J=7.2, ArH-4′), 7.57 (1H, dd, J=8.0, J=1.3, ArH-3″), 7.60 (1H, dd, J=7.6, J=1.5, ArH-6″) 7.85 (2H, d, J=7.4, ArH-2′, 6′). ^{13}C NMR (150 MHz, DMSO-d_6+CCl_4, ppm) δ: 23.68 (C-7), 126.25 (C-5″), 127.32 (C-6″), 127.98 (C-3′, 5′), 129.16 (C-2′, 6′), 130.26 (C-1″), 131.99 (C-1′), 132.17 (C-3″), 132.87 (C-4′), 133.91 (C-4″), 134.05 (C-2″), 142.31 (C-6), 151.20 (C-9), 155.35 (C-3). IR, ν, cm^{-1}: 689 (C-S), 1600 (C=N). ESI-MS: *m/z* calculated 326.04, found 327.04 [M+H]+.

*4.2.9. 3-(2,4-Dichlorophenyl)-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**11**).* Yield 81%, small needle-shaped beige crystals, m.p. 195-197°C, R_f=0,34. ^{1}H NMR (600 MHz, DMSO-d_6+CCl_4, ppm) δ: 4.33 (2H, s, S-C<u>H</u>$_2$), 7.43-7.45 (2H, m, ArH-3′,5′), 7.48-7.51 (2H, m, ArH-3″,4′), 7.63-7.64 (2H, m, ArH-2″,5″), 7.86-7.87 (2H, m, ArH-2′,6′). ^{13}C NMR (150 MHz, DMSO-d_6+CCl_4, ppm) δ: 23.67 (C-7), 125.07 (C-1″), 127.78 (C-5″), 128.03 (3′, 5′), 129.17 (2′,6′), 130.00 (1′), 132.06

(C-4′), 133.81 (C-6″), 134.06 (3″), 134.96 (2″), 136.77 (4″), 142.64 (C-6) 150.30 (C-9), 155.61 (C-3). IR, ν, cm^{-1}: 687 (C-S), 1595 (C=N). ESI-MS: *m/z* calculated 360.00, found 361.01 [M+H]+.

*4.2.10. 3-(p-Tolyl)-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**12**).* Yield 87 %, pale brown crystals, m.p. 204-205°C, R_f=0,37. ^{1}H NMR (600 MHz, DMSO-d_6+CCl_4, ppm) δ: 2.40 (3H, s, -C<u>H</u>$_3$) 3.99 (2H, s, S-C<u>H</u>$_2$), 7.28 (2H, d, J=8.0, ArH-3″,5″), 7.48-7.51 (2H, m, ArH-3′,5′), 7.55 (1H, t, J=7.3, ArH-4′), 7.87-7.89 (2H, m, ArH-2′,6′), 7.97 (2H, d, J=8.2, ArH-2″,6″). ^{13}C NMR (150 MHz, DMSO-d_6+CCl_4, ppm) δ: 21.58 (<u>C</u>H$_3$-), 23.34 (C-7), 123.38 (C-1″), 127.39 (C-2″,6″), 128.28 (C-3′, 5′), 129.27 (C-3″,5″), 129.39 (C-2′,6′), 132.06 (C-1′), 133.77 (C-4′), 140.60 (C-6), 141.68 (C-4″), 152.91 (C-9), 153.72 (C-3). IR, ν, cm^{-1}: 692 (C-S), 1612 (C=N). ESI-MS: *m/z* calculated 306.09, found 307.10 [M+H]+.

4.2.11. X-ray structural study. Crystals of 1-phenyl-2-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)ethan-1-one (**5**) were grown from ethanol by slow evaporation of the solvent at room temperature. The unit cell parameters were determined and refined on a CCD Xcalibur Ruby diffractometer (Oxford Diffraction) using CuKα radiation (T=297 K). Monoclinic system, space group $P2_1/c$, a=10.103(2), b=8.7569(18), c=15.839(3) Å, β=95.67(3)°, V=1394.4(5) Å^3, M=296.34, Z=4, $d_{calc.}$=1.412 g/cm^3, μ = 2.114, scan area $4.39 \leq \theta \leq 76.2^o$, crystal dimensions 0.45 x 0.25 x 0.20 mm. A three-dimensional set of 12561 reflections (2894 independent) was obtained. The absorption correction was introduced using the SADABS program.[42] The crystal studied was refined as an inversion twin with matrix [1 0 0, 0 -1 0, 0 0 -1].

The structure was solved by direct methods using the SHELXS-97 software package,[43] the structure refinement calculations were performed using the SHELXL-2018/3 program.[44] All non-hydrogen atoms were refined by the method of least squares in F^2 in the full-matrix anisotropic approximation to R_1=0.0615 and wR_2=1936 for 2477 reflections (goodness of fit S 1.105). The positions of hydrogen atoms were established geometrically and refined with fixed parameters of isotropic displacement $U_{iso}=nU_{eq}$, where n=1.2 for methylene groups and an aromatic ring (U_{eq} is the equivalent isotropic displacement parameter of the corresponding carbon atoms).

The results of the single crystal X-ray diffraction analysis have been deposited with the Cambridge Structural Database as a CIF-file (CCDC 2310927).

4.3. In vitro cytotoxic assay.

Epithelial carcinoma of the cervix *HeLa*, breast carcinoma cell line *HBL-100* (*ATCC NTV 124*) and adenocarcinoma of the larynx *HEp-2* (*ATCC:CCL-23*) were obtained from the Central Bank of the Collection of Cell Cultures of the Institute of Cancer Research Center of the Russian Academy of Sciences, T-lymphoblastic leukemia *CCRF-CEM* (*ATCC:CCL-19*) from the University of Heidelberg, Germany. Cell lines were cultured in RPMI-1640 and DMEM/F12 media (Capricorn scientific, Germany) with the addition of 10% bovine fetal serum (Sigma, USA) and 1x antibiotic-antimycotic solution (Lonza, Belgium).

The substances were dissolved in DMSO (0.8% by volume) and added to the cells at a concentration of 100 μM. Incubation of cells with substances was continued for 24 hours. Next, 20 μl of MTT solution (5 mg/ml) (Acros organics, Belgium) was added to the cells and left for 3-4 h. After which the wells were emptied, 50 μl of DMSO was added. Optical density was determined at 630 nm. Cell viability was determined by the ratio of living cells exposed to the test substance to the number of living cells in the control. The standard of comparison was the well-known antitumor drug cisplatin.[45]

4.4. Antibacterial and antifungal activity.

A modified agar diffusion method was used to determine antimicrobial activity.[46] The following strains of microorganisms were used as test microorganisms: *Bacillus subtilis* (RKMUz-5), *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (RKMUz-221), *Pseudomonas aeruginosa* (ATCC-27879) and *Candida albicans* (RKMUz-247). The RKMUz strains were obtained from the microorganism cultures collection of the Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan.

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