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Synthesis and antimicrobial activity of [1,2,4]triazino[2,3-c]quinazoline – pyrazoline hybrids

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ABSTRACT: Present manuscript describes the design, synthesis and antimicrobial activity of novel [1,2,4]triazino[2,3-*c*]quinazoline – pyrazoline hybrids. The combination of pyrazoline cycle with [1,2,4]triazino[2,3-*c*]quinazoline heterocyclic fragment was substantiated as promising approach for development of novel antibacterial and antifungal agents. The simple and effective synthetic procedure for target heterocyclic hybrids was proposed. The structures of obtained compounds were verified by appropriate physicochemical methods, the features of ^{1}H NMR-spectral characteristics were described as well. It was shown that synthesized compounds reveal significant growth inhibitory activity against *Candida albicans* strain.

KEYWORDS: quinazoline; pyrazoline; heterocyclic hybrids; antimicrobial activity; *Candida albicans*.

1. INTRODUCTION

Pyrazoline derivatives are well known as privileged objects for studies aimed to the search of the novel biologically active compounds [1, 2]. Approaches for their synthesis are simple and enable obtaining of the large combinatorial libraries of target compounds for screening of their biological activity. Numerous pyrazolines have been described as prominent pharmacologically valued agents with various types of effects. Thus, 3,5-diaryl-4,5-dihydro-1*H*-pyrazoles were identified as inhibitor of oxidases including monoamine oxidase [3, 4], xanthine oxidase [5] and tyrosinase [6]. The anticancer activity was reported for pyrazoline-containing molecules, including 3-[3,5-diarylpyrazol-1-yl]-2,3-dihydro-1*H*-indol-2-ones [7], isatin containing 3,5-diaryl N-acetyl pyrazolines, 2-pyrazoline-substituted 4-thiazolidinones [8, 9], 6-pyrazolinylcoumarines [10]. Pyrazoline derivatives were also described as promising antimicrobial agents. Thus, Havrylyuk and co-authors estimated the trypanocidal activity of 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones and 5-{[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxo-ethylamino]-methylene}-3-methyl-2-thioxothiazolidin-4-ones [11]. Pathak et al described the anti-tubercular activity of 3,5-diaryl-4,5-dihydro-1*H*-pyrazoles [12]. Pyrazoline derivatives with other types of biological activity were reported as well [13]. It should be noted that combining of pyrazoline fragment with other heterocycles proved to be one of the most rational approaches for design of biologically active molecules (Figure 1) [5, 7-11, 14].

In view of reasonability of abovementioned way for formation of bioactive molecules and reported by our group data about high antibacterial and antifungal activity of [1,2,4]triazino[2,3-*c*]quinazolines [15, 16] it was decided to synthesize [1,2,4]triazino[2,3-*c*]quinazoline – pyrazoline hybrids and evaluate their antimicrobial effects.

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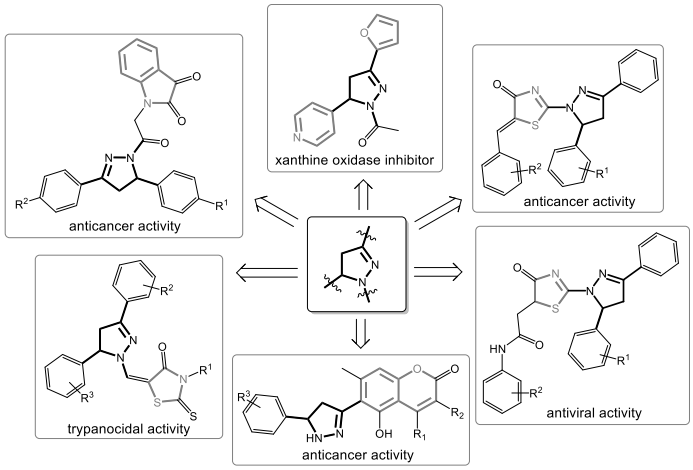

Figure 1. Biologically active pyrazoline-based heterocyclic hybrids.

2. RESULTS AND DISCUSSION

Compounds **2.1-2.12** were synthesized by alkylation of potassium thiolates **1** [16] with corresponding 1-(chloroacetyl)-3-aryl(heteryl)-5-aryl-4,5-dihydro-1*H*-pyrazoles (Figure 2). The last ones were obtained according to the described approaches *via* series of chemical transformations that included condensation of methylaryl(heteryl)ketones with aromatic aldehydes that yielded chalcones, synthesis of 3-aryl(heteryl)-5-aryl-4,5-dihydro-1*H*-pyrazoles by reaction of the obtained chalcones with hydrazine hydrate and acylation of abovementioned compounds by chloroacetyl chloride [9].

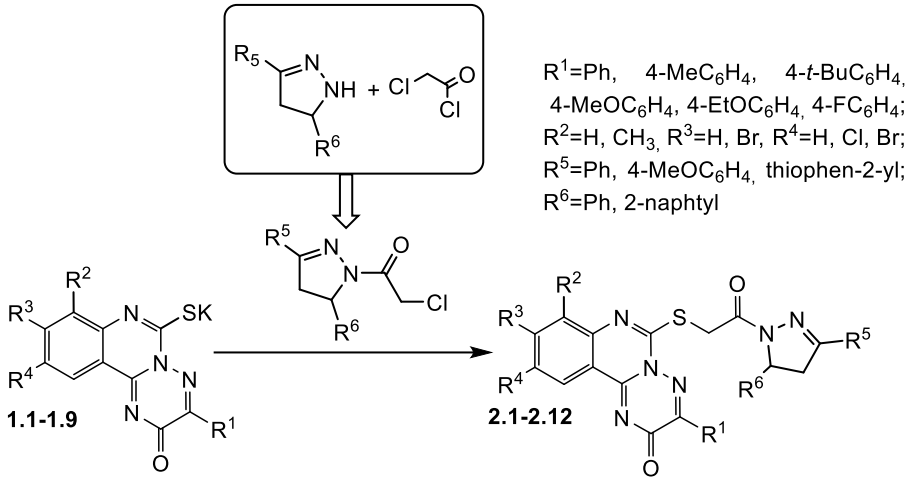

Figure 2. Synthesis of target [1,2,4]triazino[2,3-c]quinazoline – pyrazole hybrids.

The purity and structure of synthesized compounds were proven by complex of physicochemical and spectrophotometric methods including elemental analysis, LC-MS, ^{1}H NMR-spectrometry.

Signals of molecular ions with m/z values that correspond to the proposed structure were observed in LC-MS spectra of compounds **2.1-2.12**.

The ^{1}H NMR spectra of compounds **2.1-2.12** were characterized by the ABM-system of signals that corresponded to pyrazoline fragment. Abovementioned group of signals was observed as two doublets of doublets or doublets at the 3.98-3-93 ppm and 3.28-3.18 ppm with *J*=17.5-16.4 *Hz* that associated with non-equivalent methylene protons and signal of the proton at asymmetric carbon atom at the 5.65 – 5.59 ppm (*J*=11.4-9.7 *Hz*). The signals of the SCH_2CO-fragment were registered as two doublets at the 4.83-4.36 ppm and 4.53-3.93 ppm due to the presence of asymmetric carbon atom in pyrazoline fragment [17]. In ^{1}H NMR-spectra of compounds **2.1-2.5** signals of the phenyl-moieties in 3rd and 5th position of pyrazoline cycle were observed as series of multiplets that consist of two two-proton doublets (protons at 2nd and 6th positions of phenyl moieties) and two three-proton multiplets (protons at 3rd, 4th and 5th positions of phenyl moieties). At the same time signals of naphthalene protons were observed as complex multiplet at the 8.24-7.40 ppm in ^{1}H NMR-

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spectra of compound **2.6**. In ^{1}H NMR-spectra of compound **2.8** the signal of proton at the 5th position of pyrazoline ring was overlapped on the signal of proton in the 10th position of triazinoquinazoline system and observed as multiplet at the 7.66-7.59 ppm. The other signals of thiophene fragment were observed as doublets at the 7.42 ppm (3rd position) and triplet at the 7.12 ppm (4th position). In the ^{1}H NMR-spectra of compounds **2.7**, **2.9**-**2.12** signals of thiophene and signals of protons in positions 9 and 10 overlaped with each other to form multiplets. It should be noted that ^{1}H NMR -spectra of all synthesized compounds were characterized by the signals that corresponded to the benzene fragment of [1,2,4]triazino[2,3-*c*]quinazoline system and substituent an 3rd position.

Microbiological study revealed that synthesized compounds were inactive or low active against *E. coli* and *St. aureus*. Only compound **2.5** revealed moderate bacteriostatic activity against *St. aureus*. Compounds **2.1**, **2.3**, **2.4** and **2.8** reveal bacteriostatic activity against *P. aeruginosa* in concentration 50 μg/ml. It should be noted that compound **2.5** in the abovementioned concentration revealed bactericidal activity as well. At the same time most of the obtained derivatives demonstrated antifungal activity against C. albicans strain. Thus compounds **2.1**, **2.2**, **2.7**, **2.8**, **2.9**, **2.10** and **2.11** revealed fungistatic activity in the concentration 25 μg/ml and fungicide activity in the concentration 50 μg/ml.

Table 1. Antibacterial activity of synthesized compounds (**2.1-2.12**).

Compounds	*E. coli*		*St. aureus*		*P. aeruginosa*		*C. albicans*	
	MIC, μg/ml	MBC, μg/ml	MIC, μg/ml	MBC, μg/ml	MIC, μg/ml	MBC, μg/ml	MIC, μg/ml	MFC, μg/ml
2.1	200	>200	100	200	50	100	25	50
2.2	200	200	200	200	200	200	25	50
2.3	100	200	200	200	50	100	100	200
2.4	100	200	200	200	50	50	100	200
2.5	100	200	50	100	200	200	50	100
2.7	100	200	200	200	100	200	25	50
2.8	200	200	200	200	50	100	25	50
2.9	200	200	100	200	200	200	25	50
2.10	200	200	200	200	100	200	25	50
2.11	100	200	100	200	200	200	25	50
2.12	100	200	200	200	100	200	100	100
Trimetoprim	50	50	31.2	62.5	62.5	125	62.5	125
Nitrofural	1.5	–	6.25	–	6.25	–	25.0	–

3. CONCLUSION

The simple and effective method for synthesis of [1,2,4]triazino[2,3-*c*]quinazoline – pyrazoline hybrids was proposed and used for synthesis of promising antimicrobial agents. Obtained compounds in most of cases were not active against *E. coli*, *St. aureus* and *P. aeruginosa* strains. Only compound **2.5** slightly inhibited the growth of *St. aureus*, compounds **2.3**, **2.4**, **2.8** revealed low bacteriostatic effect against *P. aeruginosa*. Most of obtained compounds showed significant fungistatic activity against *C. albicans*

4. MATERIALS AND METHODS

4.1. Chemical part

4.1.1. Physicochemical medods description

Melting points were evaluated in open capillary tubes in a Stuart SMP30 (Cole-Parmer, Staffordshire, UK) device. The elemental analyses (C, H, and N) were conducted on a ELEMENTAR vario EL Cube analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany). IR spectra (4000-600 cm^{-1}) were registered using Bruker ALPHA FTIR spectrometer (Bruker Bioscience, Germany) with module for measuring attenuated total reflection. ^{1}H NMR spectra (400 MHz) were recorded on Varian-Mercury 400 (Varian Inc., Palo Alto, CA, USA) spectrometers with TMS as internal standard in DMSO-*d6* solution. LC–MS spectra were registered by chromatography/mass spectrometric system that consists of high performance liquid chromatograph Agilent 1100 Series (Agilent, Palo Alto, CA, USA) equipped with diode-matrix and mass-selective detector Agilent LC/MSD SL (atmospheric pressure chemical ionization). Compounds **1** and 1-(chloroacetyl)-3-aryl(heteryl)-5-aryl-4,5-dihydro-1*H*-pyrazoles were obtained according to the described synthetic protocols [9, 16]. The

other starting reagents and solvents were obtained from commercially available sources and were used without further purification.

4.1.2. Synthetic procedure

*General method for synthesis N^1-(3-R^5,5-R^6-pyrazol-1-yl)-2[(3-R^1-8-R^2-9-R^3-10-R^4-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl]thio)acetamides (**2.1-2.12**).*

To the suspension of 10 mmol of potassium 3-R^1-8-R^2-9-R^3-10-R^4-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-thiolate in 20 ml of dioxane or dioxane-water mixture (2:1) 11 mmol of corresponding N^1-(3-R^5-5-R^6-pyrazol-1-yl)-2-chloroacetamides was added. The formed mixture was refluxed for 60-90 min. After completion of the reaction the reaction mixture was poured into the water, formed precipitate was filtered off and dried. The obtained compounds can be crystallized from DMF.

3-(4-(*tert*-butyl)phenyl)-6-((2-(3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl)thio)-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**2.1**) (CAS number: 1787859-14-6); Yield: 89.9%; m.p.: 265-267°C; IR (cm^{-1}): 3068 , 2959, 1666 (C=O), 1632, 1588, 1562, 1549, 1500, 1471, 1451, 1434, 1372, 1342, 1312, 1289, 1274, 1243, 1227, 1198, 1160, 1144, 1126, 1107, 1078, 1042, 1024, 994, 943, 917, 885, 860, 849, 785, 769, 761, 751, 720, 701, 690, 623, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.38 (s, 9H, $C(CH_3)_3$), 3.23 (d, *J* = 16.5 *Hz*, 1H, pyr. H-4), 3.97 (dd, *J* = 16.4, 12.8 *Hz*, 1H, pyr. H-4), 4.51 (d, *J* = 16.5 *Hz*, 1H, S-CH_2), 4.81 (d, *J* = 16.5 *Hz*, 1H, S-CH_2), 5.64 (dd, *J* = 9.7, 2.5 *Hz*, 1H, pyr. H-5), 7.32 – 7.10 (m, 6H, pyr. 3 Ph H-3,4,5, pyr. 5 Ph H-3,4,5), 7.45 (d, 2H, pyr. 5 Ph H-2,6), 7.52 (d, *J* = 7.4 *Hz*, 2H, 3-Ph H-3, 5), 7.63 (t, *J* = 7.6 Hz, 1H, H-10), 7.69 (d, *J* = 7.6 Hz, 1H, H-8), 8.00 – 7.80 (m, 3H, H-9, pyr. 3 Ph H-2,6), 8.29 (d, *J* = 7.4 *Hz*, 2H, 3-Ph H-2,6), 8.54 (d, *J* = 6.6 *Hz*, 1H, H-11); LC-MS (*m/z*) =625; Calculated for: $C_{37}H_{32}N_6O_2S$: C, 71.13; H, 5.16; N, 13.45; S, 5.13; Found: C, 71.10; H, 5.11; N, 13.51; S, 5.12.

6-((2-(3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl)thio)-3-(4-ethoxyphenyl)-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**2.2**); Yield: 90.2%; m.p.: 279-281 °C; IR (cm^{-1}): 1673 (C=O), 1660, 1632, 1589, 1563, 1546, 1498, 1471, 1452, 1433, 1393, 1371, 1341, 1321, 1289, 1274, 1255, 1238, 1228, 1176, 1159, 1142, 1120, 1080, 1041, 1025, 992, 942, 921, 879, 862, 839, 799, 785, 763, 752, 720, 690, 649, 641, 621; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (s, 3H, OCH_2CH_3), 3.23 (dd, *J* = 17.6, 4.1 *Hz*, pyr. H-4), 3.98 (dd, *J* = 17.6, 4.1 *Hz*, pyr. H-4), 4.14 (q, 2H, OCH_2CH_3), 4.51 (d, *J* = 17.0 *Hz*, 1H, S-CH_2), 4.82 (d, *J* = 17.0 *Hz*, 1H, S-CH_2), 5.63 (dd, *J* = 11.4, 4.0 *Hz*, 1H, pyr. H-5), 7.91 – 6.90 (m, 16H, H-8, 9, 10, 3 Ph H-3, 5, pyr. 3 Ph H-2, 3, 4, 5, 6, pyr. 5 Ph H-2, 3, 4, 5, 6), 8.40 (d, *J* = 8.0 *Hz*, 2H, 3-Ph H-2,6), 8.54 (d, *J* = 7.7 *Hz*, 1H, H-11); LC-MS (*m/z*) =613; Calculated for: $C_{35}H_{28}N_6O_3S$: C, 68.61; H, 4.61; N, 13.72; S, 5.23; Found: C, 68.57; H, 4.55; N, 13.76; S, 5.20.

6-((2-(3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl)thio)-3-(4-fluorophenyl)-2*H*-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.3**); Yield: 85.0%; m.p.: 275-277°C; IR (cm^{-1}): 3067, 1681, 1666 (C=O), 1591, 1566, 1555, 1510, 1500, 1470, 1433, 1370, 1340, 1319, 1287, 1270, 1232, 1156, 1140, 1102, 1080, 1042, 1014, 992, 942, 882, 866, 846, 786, 768, 752, 716, 697, 689, 639, 622 ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.22 (dd, *J* = 17.3, 4.1 *Hz*, 1H, H-4), 3.97 (dd, *J* = 17.4, 12.0 Hz, 1H, H-4), 4.53 (d, *J* = 16.3 Hz, 1H, S-CH_2), 4.83 (d, *J* = 16.4 Hz, 1H, S-CH_2), 5.64 (dd, *J* = 11.4, 4.0 *Hz*, 1H, H-5), 8.05 – 6.96 (m, 15H, H-8,9,10, 3 Ph H-3,5, 3 Ph H-2, 3, 4, 5, 6, 5 Ph H-2, 3, 4, 5, 6,), 8.70 – 8.35 (m, 2H, H-11, 3 Ph H-2, H-6) ; LC-MS (*m/z*) =587; Calculated for: $C_{33}H_{23}FN_6O_2S$: C, 67.56; H, 3.95; N, 14.33; S, 5.46; Found: C, 67.61; H, 3.91; N, 14.36; S, 5.41.

6-((2-(3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl)thio)-8-methyl-3-phenyl-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**2.4**) (CAS number: 1798749-89-9); Yield: 82.8%; m.p.: 265-267°C; IR (cm^{-1}): 3065, 2939, 1667 (C=O), 1588, 1568, 1551, 1505, 1475, 1455, 1429, 1387, 1342, 1311, 1278, 1266, 1255, 1219, 1184, 1155, 1136, 1093, 1078, 1042, 1026, 1002, 986, 916, 891, 862, 830, 813, 766, 756, 716, 689, 653, 618 ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.58 (s, 3H, CH_3), 3.24 (dd, *J* = 17.6, 4.1 *Hz*, 1H, pyr. H-4), 3.93 (dd, *J* = 17.6, 4.1 *Hz*, 1H, pyr. H-4), 4.70 (dd, *J* = 18.6 *Hz*, 2H, S-CH_2), 5.65 (dd, *J* = 11.4, 4.0 *Hz*, 1H, pyr. H-5), 7.88 – 7.04 (m, 16H, H-9, 10, 3 Ph H-3, 4, 5, pyr. 3 Ph H-2, 3, 4, 5, 6, pyr. 5 Ph H-2, 3, 4, 5, 6,), 8.50 – 8.17 (m, 3H, H-11, 3-Ph H-2,6); LC-MS (*m/z*) =583; Calculated for: $C_{34}H_{26}N_6O_2S$: C, 70.09; H, 4.50; N, 14.42; S, 5.50; Found: C, 70.07; H, 4.44; N, 14.48; S, 5.47.

9-bromo-6-((2-(3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl)thio)-3-(4-fluorophenyl)-2*H*-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.5**); Yield: 85.7%; m.p.: 294-296 °C; IR (cm^{-1}): 1680, 1667 (C=O), 1583, 1561, 1499, 1460, 1427, 1371, 1338, 1313, 1296, 1274, 1234, 1185, 1158, 1113, 1102, 1077, 1061, 1042, 1016, 990, 941, 900, 866, 848, 824, 791, 768, 752, 714, 692, 680 ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.23 (dd, *J* = 17.6, 4.1 *Hz*, 1H, pyr. H-4), 3.97 (dd, *J* = 17.6, 4.1 *Hz*, 1H, pyr. H-4), 4.52 (dd, *J* = 18.6 *Hz*, 1H, S-CH_2), 4.75 (dd, *J* = 18.6 *Hz*, 1H, S-CH_2), 5.64 (dd, *J* = 11.4, 4.0 *Hz*, 1H, pyr. H-5), 8.01 – 6.54 (m, 16H, H-8, 10, 3 Ph H-3, 4, 5, pyr. 3 Ph H-2, 3, 4, 5, 6, pyr. 5 Ph H-2, 3, 4, 5, 6), 8.71 – 8.17 (m, 3H, H-11, 3-Ph H-2,6); LC-MS (*m/z*) =665; Calculated for: $C_{33}H_{22}BrFN_6O_2S$: C, 59.55; H, 3.33; N, 12.63; S, 4.82 Found: C, 59.52; H, 3.36; N, 12.68; S, 4.80.

10-chloro-6-((2-(3-(4-methoxyphenyl)-5-(naphthalen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl)thio)-3-phenyl-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**2.6**); Yield: 75.0%, m.p.: 286-288°C; IR (cm^{-1}): 1672 (C=O), 1654, 1647, 1636, 1587, 1558, 1533, 1508, 1488, 1472, 1457, 1436, 1419, 1395, 1368, 1332, 1315, 1282, 1247, 1225, 1180, 1140, 1087, 1031, 1002, 989, 954, 914, 888, 871, 853, 833, 813, 774, 744, 712, 687, 647, 617 ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.09 (d, *J* = 16.5 *Hz*, 1H, pyr. H-4), 4.53 (d, *J* = 16.5 *Hz*, 1H, S-35 (d, *J* = 16.5 *Hz*, 1H, pyr. H-4), 5.61 (dd, *J* = 9.7, 2.5 *Hz*, 1H, pyr. H-5), 6.80 (d, 2H, pyr. 5 Ph, H-2, 6), 7.18 (d, 2*H*, pyr. 5 Ph, H-2, 6), 8.24-7.40 (m, 12H, H-8, 9, 3 Ph H-3, 4 ,5, naphtalene H-1, 3, 4, 5, 6, 7, 8), 8.46 (s, 1H, H-11), 8.35 (d, 2H, 3 Ph H-2,6) ; LC-MS (*m/z*) =683; Calculated for: $C_{38}H_{27}ClN_6O_3S$: C, 66.81; H, 3.98; N, 12.30; S, 4.69; Found: C, 66.78; H, 3.96; N, 12.34; S, 4.65.

6-((2-oxo-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethyl)thio)-3-phenyl-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**2.7**); Yield: 94.3%; m.p.: 278-279 °C; IR (cm^{-1}): 3096, 3083, 3069, 3036, 2928, 1679, 1664 (C=O), 1590, 1564, 1506, 1487, 1470, 1452, 1408, 1370, 1359, 1339, 1322, 1312, 1288, 1268, 1246, 1228, 1180, 1157, 1140, 1104, 1081, 1027, 1002, 990, 967, 941, 888, 873, 859, 838, 813, 787, 773, 754, 720, 702, 690, 653, 612, ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.28 (dd, *J* = 17.6, 4.1 *Hz*, 1H, pyr. H-4), 3.93 (dd, *J* = 17.5 *Hz*, 12.0 Hz, 1H, pyr. H-4), 4.44 (d, *J* = 16.1 *Hz*, 1H, S-CH_2), 4.74 (d, *J* = 16.1 *Hz*, 1H, S-CH_2), 5.60 (dd, *J* = 11.4, 4.0 *Hz*, 1H, pyr. H-5), 7.69 – 6.56 (m, 10H, H-8, H-10, 3 Ph H-3,5, pyr. 3 Ph H-2, 3, 4, 5, 6, thioph H-3, 4, 5), 7.84 (t, *J* = 7.4 *Hz*, 1H, H-9), 8.29 (d, *J* = 8.2 *Hz*, 2H, 3-Ph H-2,6), 8.53 (d, 1H, H-11); LC-MS (*m/z*) =575, Calculated for: $C_{31}H_{22}N_6O_2S_2$: C, 64.79; H, 3.86; N, 14.62; S, 11.16; Found: C, 64.76; H, 3.84; N, 14.67; S, 11.12.

3-(4-(*tert*-butyl)phenyl)-6-((2-oxo-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethyl)thio)-2*H*-[1,2,4]triazino[2,3-c]quinazolin-2-one (**2.8**); Yield: 91.6%; m.p.: 262-263 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.38 (s, 9H, $C(CH_3)_3$), 3.23 (dd, *J* = 17.6, 4.1 *Hz*, 1H, pyr. H-4), 3.97 (dd, *J* = 17.5, 12.0 *Hz*, 1H, pyr. H-4), 4.46 (d, *J* = 16.1 *Hz*, 1H, S-CH_2), 4.76 (d, *J* = 16.1 Hz, 1H, S-CH_2-, 5.64 (dd, *J* = 11.4, 4.0 *Hz*, 1H, pyr. H-5), 7.12 (t, *J* = 3.6 *Hz*, 1H, thioph H-3), 7.31 – 7.15 (m, 5H, pyr. 3 Ph H-2, 3, 4, 5, 6), 7.42 (d, *J* = 2.3 *Hz*, 1H, thioph H-4), 7.52 (d, *J* = 8.1 *Hz*, 2H, 3 Ph H-3,5), 7.66 – 7.59 (m, 2H, H-10, thioph H-5), 7.68 (d, *J* = 7.9 *Hz*, 1H, H-8), 7.89 (t, *J* = 7.4 *Hz*, 1H, H-9), 8.29 (d, *J* = 8.2 *Hz*, 2H, 3-Ph H-2,6), 8.53 (d, *J* = 7.8 *Hz*, 1H, H-11) ; LC-MS (*m/z*) =631; Calculated for: $C_{35}H_{30}N_6O_2S_2$: C, 66.64; H, 4.79; N, 13.32; S, 10.17; Found: C, 66.61; H, 4.82; N, 13.27; S, 10.12.

3-(4-ethoxyphenyl)-6-((2-oxo-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethyl)thio)-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**2.9**); Yield: 88.4%; m.p.: 264-266°C; IR (cm^{-1}): 3072, 3037, 2961, 2871, 1665 (C=O), 1589, 1549, 1526, 1499, 1472, 1454, 1415, 1372, 1343, 1323, 1289, 1275, 1245, 1227, 1197, 1173, 1160, 1143, 1109, 1079, 1024, 996, 976, 965, 944, 887, 867, 849, 838, 800, 786, 769, 752, 745, 701, 689, 639, 624, 612, ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (s, 3H, OCH_2CH_3), 3.23 (d, *J* = 18.3 *Hz*, 1H, pyr. H-4), 3.98 (d, *J* = 18.3 *Hz*, 1H, pyr. H-4), 4.12 (q, 2H, OCH_2CH_3), 4.45 (d, *J* = 16.2 *Hz*, 1H, S-CH_2), 4.76 (d, *J* = 16.2 *Hz*, 1H, S-CH_2), 5.62 (dd, *J* = 11.4, 4.0 *Hz*, 1H, pyr. H-5), 7.78 – 6.89 (m, 10H, H-8, H-10, 3 Ph H-3,5, pyr. 3 Ph H-2, 3, 4, 5, 6, thioph H-3, 4, 5), 7.88 (t, *J* = 7.7 *Hz*, 1H, H-9), 8.38 (d, *J* = 8.0 *Hz*, 2H, 3-Ph H-2,6), 8.48 (d, *J* = 7.7 *Hz*, 1H, H-11) ; LC-MS (*m/z*) =619; Calculated for: $C_{33}H_{26}N_6O_3S_2$: C, 64.06; H, 4.24; N, 13.58; S, 10.36; Found: C, 64.02; H, 4.28; N, 13.64; S, 10.31

3-(4-fluorophenyl)-6-((2-oxo-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethyl)thio)-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**2.10**); Yield: 84.2%; m.p.: 280-282°C; IR (cm^{-1}): 3074, 3039, 1679, 1664 (C=O), 1626, 1590, 1566, 1554, 1497, 1470, 1455, 1415, 1366, 1340, 1322, 1287, 1270, 1228, 1158, 1139, 1104, 1080, 1030, 1014, 992, 967, 943, 889, 873, 847, 839, 785, 768, 753, 743, 699, 688, 640, 624, 617 ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.25 (d, *J* = 16.5 *Hz*, 1H, pyr. H-4), 3.97 (d, *J* = 16.5 *Hz*, 1H, pyr. H-4), 4.50 (d, *J* = 16.5 *Hz*, 1H, S-CH_2), 4.75 (, *J* = 16.5 *Hz*, 1H, S-CH_2), 5.62 (dd, *J* = 9.7, 2.5 *Hz*, 1H, pyr. H-5), 7.46- 6.98 (m, 8H, H-8, 10, thioph H-4, pyr. 3 Ph H-2, 3, 4, 5, 6), 7.76 – 7.56 (m, 2H, thioph H-3, 5), 7.90 (t, *J* = 7.6 *Hz*, 1H, H-9), 8.44 (d, *J* = 7.4 *Hz*, 2H, 3-Ph H-2,6), 8.54 (d, *J* = 7.6 *Hz*, 1H, H-11) ; LC-MS (*m/z*) = 593; Calculated for: $C_{33}H_{26}N_6O_3S_2$; C, 62.82; H, 3.57; N, 14.18; S, 10.82; Found: C, 62.84; H, 3.61; N, 14.23; S, 10.80.

10-chloro-6-((2-oxo-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethyl)thio)-3-(p-tolyl)-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**2.11**); Yield: 67.2%; m.p.: 291-293 °C; IR (cm^{-1}): 2961, 2921, 2854, 1672 (C=O), 1586, 1556, 1501, 1469, 1455, 1415, 1369, 1340, 1324, 1284, 1265, 1228, 1186, 1139, 1121, 1087, 1025, 995, 958, 893, 848, 834, 773, 758, 742, 714, 701, 667, 641, 629, 615 ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.40 (s, 3H, CH_3), 3.18 (dd, *J* = 17.6, 4.1 *Hz*, 1H, pyr. H-4), 3.87 (dd, *J* = 17.5, 12.0 *Hz*, 1H, pyr. H-4), 3.93 (d, *J* = 16.1 *Hz*, 1H, S-CH2), 4.36 (d, *J* = 16.1 Hz, 1H, S-CH_2), 5.88 – 5.18 (dd, *J* = 11.4, 4.0 *Hz*, 1H, pyr. H-5), 7.40 – 6.96 (m, 7H, pyr. 3 Ph H-2, 3, 4, 5, 6, thioph H-3, 4), 7.96 – 7.76 (m, 2H, H-8, thioph H-5), 7.59 (d, *J* = 8.1 *Hz*, 2H, 3 Ph H-3,5), 8.10 (t, *J* = 7.4 Hz, 1H, H-9), 8.24 (d, *J* = 8.2 *Hz*, 2H, 3-Ph H-2,6), 8.41 (d, 1H, H-11); LC-MS (*m/z*) =623; Calculated for: $C_{32}H_{23}ClN_6O_2S_2$: C, 61.68; H, 3.72; N, 13.49; S, 10.29; Found: C, 61.71; H, 3.77; N, 13.54; S, 10.33

10-bromo-3-(4-fluorophenyl)-6-((2-oxo-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethyl)thio)-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**2.12**); Yield: 71.7%; m.p.: 287-289°C; IR (cm^{-1}): 3091, 3036, 3013, 2975, 2930, 1670 (C=O), 1591, 1557, 1499, 1459, 1419, 1369, 1327, 1306, 1290, 1279, 1260, 1228, 1213, 1160, 1142, 1123, 1105, 1080, 1030, 1012, 989, 953, 906, 891, 869, 851, 839, 823, 800, 786, 769, 757, 743, 729, 697, 655, 636, 625, 615 1H NMR (400 MHz, DMSO-d_6) δ 3.42 – 3.07 (dd, *J* = 17.6, 4.1 *Hz*, 1H, pyr. H-4), 3.96 (dd, *J* = 17.6, 4.1 *Hz*, 1H, pyr. H-4), 4.47 (d, *J* = 15.9 *Hz*, 1H, S-CH_2), 4.75 (d, *J* = 15.9 *Hz*, 1H, S-CH_2), 5.59 (dd, *J* = 11.4, 4.0 *Hz*, 1H, pyr. H-5), 7.36 – 6.91 (m, 6H, 3 Ph H-2, 3, 4, 5, 6, thioph H-4), 7.61 (m, 2H, H-8, thioph H-5), 7.42 (t, thioph H-3), 7.99 (t, *J* = 7.4 *Hz*, 1H, H-9), 8.44 (d, J = 8.2 Hz, 2H, 3-Ph H-2,6), 8.61 (d, 1H, H-11) ; LC-MS (*m/z*) =671; Calculated for: $C_{31}H_{20}BrFN_6O_2S_2$: C, 55.44; H, 3.00; N, 12.51; S, 9.55; Found: , 55.50; H, 3.02; N, 12.55; S, 9.59

4.2. Antimicrobial test

The sensitivity of the microorganisms to the synthesized compounds was evaluated according to the described methods [18]. The assay was conducted on Mueller-Hinton medium by two-fold serial dilution of the compound in 1 ml. After that, 0.1 ml of microbial seeding (106 cells/ml) was added. Minimal inhibit concentration of the compound was determined by the absence of visual growth in the test tube with a minimal concentration of the substance, minimal bactericide/fungicide concentration was determined by the absence of growth on agar medium after inoculation of the microorganism from the transparent test-tubes. DMSO was used as a solvent, initial solution concentration was 1 mg/ml. For preliminary screening the mentioned ahead standard test cultures were used: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 885-653 standard test cultures. All test strains were received from bacteriological laboratory in Zaporizhzhia Regional Laboratory Center of State Sanitary and Epidemiological Service of Ukraine. Nitrofural and Trimetoprim were used as reference compounds with proved antibacterial/antifungal activity. Additional quality control of the culture medium and solvents was conducted by commonly used methods [18].

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REFERENCES

[1] Nehra B, Rulhania S, Jaswal S, Kumar B, Singh G, Monga V. Recent advancements in the development of bioactive pyrazoline derivatives. Eur J Med Chem. 2020; 205: 112666. [CrossRef]

[2] Matiadis D, Sagnou M. Pyrazoline Hybrids as Promising Anticancer Agents: An Up-to-Date Overview. Int J Mol Sci. 2020; 21(15): 5507. [CrossRef]

[3] Chimenti F, Carradori S, Secci D, Bolasco A, Bizzarri B, Chimenti P, Granese A, Yanez M, Orallo, F. Synthesis and inhibitory activity against human monoamine oxidase of N1-thiocarbamoyl-3,5-di(hetero)aryl-4,5-dihydro-(1H)-pyrazole derivatives. Eur J Med Chem. 2010; 45(2): 800–804. [CrossRef]

[4] Palaska E, Aydin F, Uçar G, Erol D. Synthesis and monoamine oxidase inhibitory activities of 1-thiocarbamoyl-3,5-diphenyl-4,5-dihydro-1H-pyrazole derivatives. Arch Pharm. 2008; 341(4): 209–215. [CrossRef]

[5] Nepali K, Singh G, Turan A, Agarwal A, Sapra S, Kumar R, Dhar KL. A rational approach for the design and synthesis of 1-acetyl-3,5-diaryl-4,5-dihydro(1H)pyrazoles as a new class of potential non-purine xanthine oxidase inhibitors. Bioorg Med Chem. 2011; 19(6): 1950–1958. [CrossRef]

[6] Zhou Z, Zhuo J, Yan S, Ma L. Design and synthesis of 3,5-diaryl-4,5-dihydro-1H-pyrazoles as new tyrosinase inhibitors. Bioorg Med Chem. 2013; 21(7): 2156–2162. [CrossRef]

[7] Havrylyuk D, Zimenkovsky B, Vasylenko O, Gzella A, Lesyk R. Synthesis of new 4-thiazolidinone-, pyrazoline-, and isatin-based conjugates with promising antitumor activity. J Med Chem. 2012; 55(20): 8630–8641. [CrossRef]

[8] Sharma M, Sharma S, Buddhiraja A, Saxena AK, Nepali K, Bedi PMS. Synthesis and cytotoxicity studies of 3,5-diaryl N-acetyl pyrazoline—isatin hybrids. Med Chem Res. (2014); 23(10): 4337–4344. [CrossRef]

[9] Havrylyuk D, Kovach N, Zimenkovsky B, Vasylenko O, Lesyk R. Synthesis and anticancer activity of isatin-based pyrazolines and thiazolidines conjugates. Arch Pharm. 2011; 344(8): 514–522. [CrossRef]

[10] Garazd Y, Garazd M, Lesyk R. Synthesis and evaluation of anticancer activity of 6-pyrazolinylcoumarin derivatives. Saudi Pharm J. 2017; 25(2): 214–223. [CrossRef]

[11] Havrylyuk D, Zimenkovsky B, Karpenko O, Grellier P, Lesyk R. Synthesis of pyrazoline–thiazolidinone hybrids with trypanocidal activity. Eur J Med Chem. 2014; 85: 245–254. [CrossRef]

[12] Pathak V, Maurya HK, Sharma S, Srivastava KK, Gupta A Synthesis and biological evaluation of substituted 4,6-diarylpyrimidines and 3,5-diphenyl-4,5-dihydro-1H-pyrazoles as anti-tubercular agents. Bioorg Med Chem Lett. 2014; 24(13): 2892–2896. [CrossRef]

[13] Alex JM, Kumar R. 4,5-Dihydro-1H-pyrazole: an indispensable scaffold. J Enzyme Inhib Med Chem. 2013; 29(3): 427–442. [CrossRef]

[14] Havrylyuk D, Zimenkovsky B, Vasylenko O, Lesyk R. Synthesis and Anticancer and Antiviral Activities of New 2-Pyrazoline-Substituted 4-Thiazolidinones. J Heterocycl Chem. 2013; 50(S1): E55–E62. [CrossRef]

[15] Berest GG, Voskoboynik OY, Kovalenko SI, Nosulenko IS, Antypenko LM, Antypenko OM, Shvets VM, Katsev AM. Synthesis of new 6-{[ω-(dialkylamino(heterocyclyl)alkyl]thio}-3-R-2H-[1,2,4]triazino[2,3-c]quinazoline-2-ones and evaluation of their anticancer and antimicrobial activities. Sci Pharm. 2012; 80(1): 37–65. [CrossRef]

[16] Nosulenko IS, Voskoboynik OY, Berest GG, Safronyuk SL, Kovalenko SI, Kamyshnyi OM, Polishchuk NM, Sinyak RS, Katsev AV. Synthesis and antimicrobial activity of 6-thioxo-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one derivatives. Sci Pharm. 2014; 82(3): 483–500. [CrossRef]

[17] Breitmaier E, Structure Elucidation By NMR In Organic Chemistry: A Practical Guide, John Wiley &Sons, Ltd, 2002. [CrossRef]

[18] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal 2016; 6(2): 71-79. [CrossRef]