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Synthesis, anticancer and antimicrobial properties of some *N*-aryl-2-(5-aryltetrazol-2-yl)acetamides

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A B S T R A C T

The synthesis, anticancer and antimicrobial properties of novel *N*-aryl-2-(5-aryltetrazol-2-yl)acetamides were discussed. Novel *N*-aryl-2*H*-tetrazoles were synthesized and modified in order to obtain the compounds with a satisfactory pharmacological profile. The structures of target substances were confirmed by using ^{1}H spectroscopy, mass spectrometry and elemental analysis. Anticancer activity screening was carried out within the framework of Developmental Therapeutic Program of the National Cancer Institute's (DTP, NCI, Bethesda, Maryland, USA). The compounds with significant levels of anticancer activities have been found that can be used for further optimization. The antimicrobial activity of the synthesized substances was evaluated by the value of the MIC and minimum fungicidal and bactericidal concentration. The findings exhibited that the compounds possessed moderate antimicrobial potential.

1. Introduction

Nowadays, cancer is one of the major diseases which results in mortality, and its occurrence is increasing by the year. It is considered a public health problem according to the World Health Organization, and many efforts have been made towards its prevention and cure.[1] No less interesting is the search for new antimicrobial drugs. Microbial infections become an increasingly serious and challenging problem for human health across the world. The increasing resistance to the current antimicrobial treatment has resulted in crucial need for the discovery and development of novel drugs for the infectious treatment.[2] So, in order to meet above mentioned challenges, there is an urgent need for the development of novel more effective anticancer and antimicrobial agents.

Nitrogen-containing organic molecules have acquired special attention in the field of organic, bioorganic and medicinal chemistry. They contributed to the development of numerous organic synthesis protocols and found abundant applications in the chemical sciences. Tetrazoles are an important class of nitrogen-comprising heterocycles. The heterocyclic tetrazole moiety has admirable biological, pharmaceutical and clinical applications. Among the specified class of compounds were researched antimicrobial,[3-5] anti-fungal,[6] anti-viral,[7] antitubercular,[8] antiproliferative,[9] anti-inflammatory,[10] antioxidant,[11] anticancer,[12] anti-hypertension[13] activities. Some of their analogues were recognized as novel lGIRK1/2 potassium channel activators,[14] potent pan-KIT mutant inhibitors,[15] inhibitors of Mur B,[16] inhibitors of scavenger receptor BI (SR-BI)-mediated lipid uptake[17] and inhibitors of antiapoptotic bcl-2 family proteins.[18]

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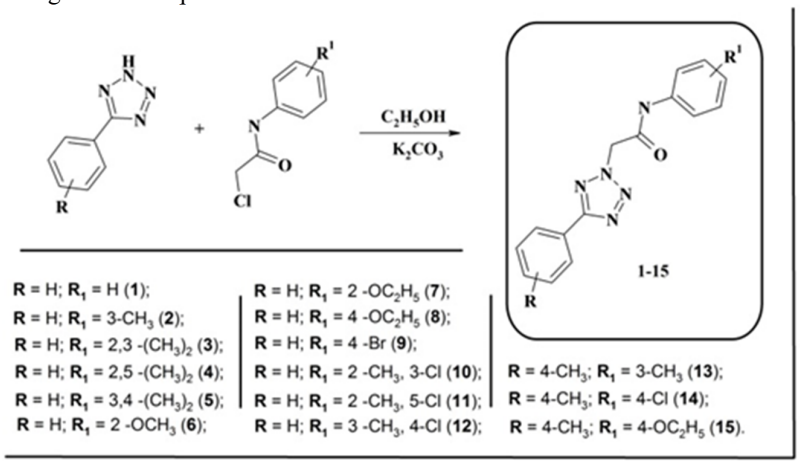
Taking all mentioned above into account, in this paper, we present work was to synthesize a series of novel *N*-aryl-2-(5-aryltetrazol-2-yl)acetamides by means with further pharmacological screening on anticancer and antimicrobial activities.

2. Results and Discussion

2.1 Chemistry

In view of continuation of our research work to find new effective biologically active azaheterocycles,[19-33] in this article we reported about of synthesis, anticancer and antimicrobial activities of *N*-aryl-2-(5-aryltetrazol-2-yl)acetamides. The desired compounds (**1-15**) were prepared by reacting 5-phenyl-2*H*-tetrazole or 5-p-tolyl-2*H*-tetrazole with appropriate 2-chloro-*N*-arylacetamides. This nucleophilic substitution reaction was carried out in the presence of potassium carbonate. This reaction is summarized in the scheme.

Obtained compounds structures were confirmed by ^{1}H NMR spectroscopy, mass spectrometry, and elemental analysis. In ^{1}H-NMR spectra, the signals for the protons of all the structural units were observed in their characteristic ranges.



Scheme.

2.2 Anticancer activity

The synthesized compounds were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) for the *in vitro* cell line screening to investigate their anticancer activity. Primary anticancer assay was performed at approximately sixty human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda (USA).[34-37] The results for each tested compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. The results of primary screening are reported as the percent of cancer cell line growth (GP %) and are presented in Table 1. The range of GP% shows the lowest and the highest values founded for different cancer cell lines.

Table 1
Cytotoxic activity of the tested compounds in the concentration 10^{-5} M against 60 cancer cell lines.

Test compounds	Range of growth, %	Most sensitive cell line (cancer line/type) GP, %
1	50.24 - 139.69	UO-31 Renal Cancer 50.24 IGROV1 Ovarian Cancer 62.57 MOLT-4 Leukemia 73.02 NCI/ADR-RES Ovarian Cancer 84.18 OVCAR-4 Ovarian Cancer 85.22 SF-268 CNS Cancer 85.93
2	88.99 - 140.37	UO-31 Renal Cancer 88.99
3	62.14 - 129.45	HOP-92 Non-Small Cell Lung Cancer 62.14 MCF7 Breast Cancer 80.97 DU-145 Prostate Cancer 82.72 HT29 Colon Cancer 84.56 NCI-H522 Non-Small Cell Lung Cancer 84.66 PC-3 Prostate Cancer 85.93
4	66.39 - 141.88	HOP-92 Non-Small Cell Lung Cancer 66.39 HL-60(TB) Leukemia 71.64

		U251 CNS Cancer 83.63 TK-10 Renal Cancer 83.20 MDA-MB-468 Breast Cancer 83.79
5	65.99 - 123.42	UO-31 Renal Cancer 65.99 IGROV1 Ovarian Cancer 76.09 UACC-257 Melanoma 82.14 MDA-MB-231/ATCC Breast Cancer 85.92
6	34.42 - 122.56	PC-3 Prostate Cancer 34.42 RPMI-8226 Leukemia 50.71 HT29 Colon Cancer 52.60 HL-60(TB) Leukemia 53.79 MDA-MB-468 Breast Cancer 57.82 K-562 Leukemia 57.83 CCRF-CEM Leukemia 59.09
7	82.51 - 113.41	HCT-116 Colon Cancer 82.51 HOP-62 Non-Small Cell Lung Cancer 84.24 SK-MEL-2 Melanoma 85.66
8	39.32 - 114.15	RXF 393 Renal Cancer 39.32 NCI-H522 Non-Small Cell Lung Cancer 45.02 IGROV1 Ovarian Cancer 45.37 HOP-92 Non-Small Cell Lung Cancer 49.42 SF-539 CNS Cancer 54.27
9	77.93 - 173.89	CCRF-CEM Leukemia 77.93 MCF7 Breast Cancer 79.70 DU-145 Prostate Cancer 83.00
10	57.28 - 118.40	T-47D Breast Cancer 57.28 SR Leukemia 59.81 MALME-3M Melanoma 64.18 UO-31 Renal Cancer 67.32 SNB-19 CNS Cancer 67.71 HOP-62 Non-Small Cell Lung Cancer 69.60
11	59.06 - 113.88	UO-31 Renal Cancer 59.06 IGROV1 Ovarian Cancer 76.43 NCI-H522 Non-Small Cell Lung Cancer 83.07
12	51.20 - 125.00	CCRF-CEM Leukemia 51.20 UO-31 Renal Cancer 71.03 HOP-92 Non-Small Cell Lung Cancer 74.41 IGROV1 Ovarian Cancer 78.25
13	70.81 - 118.45	UO-31 Renal Cancer 70.81 HL-60(TB) Leukemia 73.24 SR Leukemia 76.58 MOLT-4 Leukemia 77.12 HOP-62 Non-Small Cell Lung Cancer 78.62
14	77.37 - 105.66	K-562 Leukemia 77.37 CCRF-CEM Leukemia 77.82 SR 7 Leukemia 78.49 UO-31 Renal Cancer 79.69
15	81.97 - 128.62	UO-31 Renal Cancer 81.97

As the experiment showed, all compounds showed low or moderate activity against most malignant tumor cells. But in case of compounds **1**, **6**, **8**, **10**, **11**, **12** against several cancer cell lines the high activities was observed. The most sensitive were PC-3 Prostate cancer cell lines (GP = 34.42 %) and RXF 393 Renal cancer cell line (GP = 39.32%) to the compouds **6** and **8**. The compound **8** stimulate growing of NCI-H522 Non-Small Cell Lung Cancer, IGROV1 Ovarian Cancer and HOP-92 Non-Small Cell Lung Cancer. It should also be noted that compounds **1**, **10**, **11** and **12** effectively promote growth of UO-31 Renal Cancer cell line.

2.3 Antimicrobial activity

The antifungal and antibacterial activity of the synthesized substances was evaluated by the value of the MIC and minimum fungicidal and bactericidal concentration (MFC and MBC). Test cultures of microorganisms were fungi of the genus *Candida albicans*, as well as some gram-positive and gram-negative bacteria S. *aureus 25923* and *E. coli 25922*. The drug "Bifonazole" was used as a control (**Tables 2, 3**). Microbiological studies made it possible to compare that the that *N*-aryl-2-(5-aryl-tetrazol-2-yl)-acetamides are characterized by antimicrobial action in a wide concentration range of 62.5–500 μg/ml.[38]

The antimicrobial activity of the test compounds was evaluated by the obtained results of antimicrobial activity, which was expressed in minimal bacteriostatic, bactericidal, fungistatic and fungicidal concentrations. As can be seen from Table 2, the fungal activity against *C. albicans ATCC 885-653* of the test compounds ranged from 62.5 μg/ml to 500 μg/ml. The best results were observed in compounds 6 MFsC (83.3 μg/ml), MFcC (104.2 μg/ml), slightly lower in 15 and 8 MFsC (83.3 μg/ml, 104.2 μg/ml), MFcC - (125 μg / ml) for both.

The remaining test compounds showed antifungal activity at concentrations one order of magnitude and two orders of magnitude lower than the control (125-250 μg/ml).

The antimicrobial activity of the test compounds against the reference strain of gram-positive bacterium *S. aureus ATCC 25923* was an order of magnitude lower than the antifungal activity. Of note are compounds **14**, **12** and **15**, their MBsC and MBcC ranged from 125-250 μg / ml. Reference strain *E. coli ATCC 25922* showed the following sensitivity to the test compounds. Regarding control, the best result was observed in compound **14**, its MBsC was 125 μg/ml and MBcC 166.7 μg/ml. In most compounds, the cid effect on *E. coli ATCC 25922* was observed at concentrations an order of magnitude higher than static .

Table 2. Antifungal activity compounds **1-15**.

Test compounds or control	C. *Albicans 885-653* (microbial load - 10^3)					
	MIC			MFC		
1	250	125	250	250	250	250
2	250	250	250	250	250	250
3	125	125	125	125	250	125
4	250	250	250	250	250	250
5	250	125	125	125	125	125
6	125	62.5	62.5	125	62.5	125
7	125	125	125	125	125	125
8	125	62.5	125	125	125	125
9	125	250	250	250	250	250
10	250	250	250	250	250	250
11	250	125	125	250	250	250
12	250	125	125	250	250	250
13	125	125	125	125	125	125
14	125	125	125	125	125	125
15	62.5	125	62.5	125	125	125
control	500	500	500	500	500	500

Table 3. Antibacterial activity compounds **1-15**.

Test compounds or control	*S. aureus 25923* (microbial load – 10^5)						*E. coli 25922* (microbial load – 10^5)					
	MIC			MBC			MIC			MBC		
1	250	250	250	250	250	250	250	250	250	250	250	500
2	250	250	250	250	250	250	250	250	250	250	250	250
3	250	250	250	250	250	250	250	250	250	250	250	500
4	250	500	250	250	500	250	250	250	500	500	250	250
5	125	250	250	250	250	250	250	125	250	250	250	250
6	500	500	500	500	500	500	500	500	500	500	500	500
7	250	250	125	125	250	250	125	250	250	250	250	125
8	125	250	250	250	250	250	250	125	250	250	250	250
9	250	250	250	250	250	250	250	250	250	250	250	250
10	250	250	250	250	250	250	250	250	250	250	500	250
11	125	250	250	250	250	250	250	125	250	250	250	250
12	125	250	125	250	250	250	125	125	250	250	250	250
13	250	250	250	250	250	250	250	250	250	250	250	250
14	125	125	125	125	125	250	125	125	125	125	250	125
15	125	250	125	125	250	250	125	125	250	250	250	125
control	500	500	500	500	500	500	500	500	500	500	500	500

Results revealed that synthesized compounds have moderate antibacterial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. Additionally the derivatives **6** and **15** showed medium antifungal activity against *Candida albicans*.

3. Conclusions

In summary, we presented an efficient synthetic approaches to a number of *N*-aryl-2-(5-aryltetrazol-2-yl)acetamides for their anticancer and antimicrobial activity evaluation. The structures of aimed substances were reaffirmed by using ^{1}H-NMR spectroscopy, mass spectrometry and elemental analysis. The obtained results of the performed biological activity evaluation suggested the synthesized compounds as a promising structures in anticancer and antimicrobial drugs development. Further optimization of the structure to improve biological activity is currently in progress.

4. Experimental

4.1 Chemistry

All chemicals were of analytical grade and commercially available. When performing the synthetic part of the work, the reagents of the company Merck (Germany) and Sigma-Aldrich (USA) were used. All reagents and solvents were used without further purification and drying. All the melting points were determined in an open capillary and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian Mercury 400 (Agilent Technologies, San Francisco, USA) instrument with TMS or deuterated solvent as an internal reference. Mass spectra were run using Agilent 1100 series LC/MSD (Agilent Technologies, San Francisco, USA) with an API–ES/APCI ionization mode. Elemental analysis was performed on an Elementar Vario L cube instrument (Elementar Analysen systeme GmbH, Hanau, Germany). Satisfactory elemental analyses were obtained for new compounds (C±0.17, H±0.21, N±0.19).

4.1.1 General procedure for the synthesis of N-aryl-2-(5-aryltetrazol-2-yl)acetamides (**1-15**)**.** The solution of 0.72 g 5-phenyl-2*H*-tetrazole or 5-p-tolyl-2H-tetrazole, 5 mmol the corresponding 2-chloro-*N*-arylacetamide and 0.7 g potassium carbonate in 20 ml EtOH was refluxed for 3 hours, then allowed for cooling. The obtained precipitate was filtered, washed with EtOH, dried and crystallized from EtOH.

4.1.2 N-Phenyl-2-(5-phenyltetrazol-2-yl)acetamide (**1**). Yield 84 %, m.p. 163–164 °C. ^{1}H NMR (400 MHz, DMSO) δ = 5.68 (s, 2H, CH_2), 7.07 (t, *J* = 7.4 Hz, 1H, N-C_6H_5), 7.30 (t, *J* = 7.6, 8.4 Hz, 2H, N-C_6H_5), 7.50-7.56 (m, 3H, C_6H_5), 7.60 (d, *J* = 7.7 Hz, 2H, N-C_6H_5), 8.11 (dd, *J* = 8.2 Hz, 2H, C_6H_5), 10.56 (s, 1H, NH). ESI-MS: m/z 280 $[M+H]^+$. Anal. Calcd. for $C_{15}H_{13}N_5O$: C, 64.51 H, 4.69; N, 25.07. Found: C, 64.77; H, 4.61; N, 25.22.

4.1.3 2-(5-Phenyltetrazol-2-yl)-N-m-tolylacetamide (**2**). Yield 69 %, m.p. 170–171 °C. ^{1}H NMR (400 MHz, DMSO) δ = 2.33 (s, 3H, CH_3), 5.66 (s, 2H, CH_2), 6.88 (d, *J* = 7.5 Hz, 1H, N-C_6H_4), 7.17 (t, *J* = 7.8, 7.9 Hz, 1H, N-C_6H_4), 7.37 (d, *J* = 8.1 Hz, 1H, N-C_6H_4), 7.43 (s, 2H, N-C_6H_4), 7.50-7.56 (m, 3H, C_6H_5), 8.11 (dd, *J* = 8.1 Hz, 2H, C_6H_5), 10.46 (s, 1H, NH). ESI-MS: m/z 294 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{15}N_5O$: C, 65.52 H, 5.15; N, 23.88. Found: C, 65.65; H, 5.20; N, 23.93.

4.1.4 N-(2,3-Dimethylphenyl)-2-(5-phenyl-tetrazol-2-yl)acetamide (**3**). Yield 72 %, m.p. 186 °C. ^{1}H NMR (400 MHz, DMSO) δ = 2.18 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 5.70 (s, 2H, CH_2), 6.99-7.06 (m, 2H, N-C_6H_3), 7.22 (d, *J* = 7.5, Hz, 1H, N-C_6H_3), 7.47-7.54 (m, 3H, C_6H_5), 8.11 (d, *J* = 7.2 Hz, 2H, C_6H_5), 9.89 (s, 1H, NH). ESI-MS: m/z 308 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{17}N_5O$: C, 66.43 H, 5.58; N, 22.79. Found: C, 66.74; H, 5.61; N, 22.65.

4.1.5 N-(2,6-Dimethylphenyl)-2-(5-phenyltetrazol-2-yl)acetamide (**4**). Yield 80 %, m.p. 194 °C. ^{1}H NMR (400 MHz, DMSO) δ = 2.24 (s, 6H, 2*CH_3), 5.70 (s, 2H, CH_2), 7.02-7.09 (m, 3H, N-C_6H_3), 7.47-7.57 (m, 3H, C_6H_5), 8.12 (d, *J* = 7.1 Hz, 2H, C_6H_5), 9.84 (s, 1H, NH). ESI-MS: m/z 308 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{17}N_5O$: C, 66.43 H, 5.58; N, 22.79. Found: C, 66.40; H, 5.62; N, 22.84.

4.1.6 N-(3,4-Dimethylphenyl)-2-(5-phenyltetrazol-2-yl)acetamide (**5**). Yield 85 %, m.p. 182 °C. ^{1}H NMR (400 MHz, DMSO) δ = 2.21 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 5.64 (s, 2H, CH_2), 7.03 (d, *J* = 8.2, Hz, 1H, N-C_6H_3), 7.30 (d, *J* = 8.1 Hz, 1H, N-C_6H_3), 7.36 (s, 1H, N-C_6H_3), 7.48-7.56 (m, 3H, C_6H_5), 8.11 (dd, *J* = 8.1 Hz, 2H, C_6H_5), 10.37 (s, 1H, NH). ESI-MS: m/z 308 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{17}N_5O$: C, 66.43 H, 5.58; N, 22.79. Found: C, 66.45; H, 5.53; N, 22.71.

4.1.7 N-(2-Methoxyphenyl)-2-(5-phenyltetrazol-2-yl)acetamide (**6**). Yield 74 %, m.p. 192-193 °C. ^{1}H NMR (400 MHz, DMSO) δ = 3.92 (s, 3H, O-CH_3), 5.78 (s, 2H, CH_2), 6.99 (t, *J* = 7.1, 8.1 Hz, 1H, N-C_6H_4), 7.01-7.09 (m, 2H, N-C_6H_4), 7.50-7.56 (m, 3H, C_6H_5), 7.99 (d, *J* = 8.0 Hz, 1H, N-C_6H_4), 8.10 (d, *J* = 8.0 Hz, 2H, C_6H_5), 9.82 (s, 1H, NH). ESI-MS: m/z 310 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{15}N_5O_2$: C, 62.13 H, 4.89; N, 22.64. Found: C, 62.06; H, 4.93; N, 22.59.

4.1.8 N-(2-Ethoxyphenyl)-2-(5-phenyltetrazol-2-yl)acetamide (**7**). Yield 56 %, m.p. 211–212 °C. ^{1}H NMR (400 MHz, DMSO) δ = 1.21 (t, *J* = 7.5 Hz, 3H, O-CH_2CH_3), 2.65-2.71 (m, 2H, O-CH_2CH_3), 5.72 (s, 2H, CH_2), 7.12-7.18 (m, 2H, N-C_6H_4), 7.22-7.24 (m, 1H, N-C_6H_4), 7.41-7.44 (m, 1H, N-C_6H_4), 7.48-7.56 (m, 3H, C_6H_5), 8.10 (d, *J* = 8.1 Hz, 2H, C_6H_5),

9.88 (s, 1H, NH). ESI-MS: m/z 324 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{17}N_5O_2$: C, 63.15 H, 5.30; N, 21.66. Found: C, 63.28; H, 5.26; N, 21.72.

4.1.9 N-(4-Ethoxyphenyl)-2-(5-phenyltetrazol-2-yl)acetamide (**8**). Yield 61 %, m.p. 166–167 °C. ^{1}H NMR (400 MHz, DMSO) δ = 1.34-1.40 (m, 3H, O-$CH_2\underline{CH_3}$), 3.97-4.05 (m, 2H, O-$\underline{CH_2}CH_3$), 5.63 (s, 2H, CH_2), 6.82 (d, *J* = 9.0 Hz, 2H, N-$\underline{C_6H_4}$), 7.48-7.56 (m, 5H, C_6H_5+N-$\underline{C_6H_4}$), 8.11 (dd, *J* = 8.1 Hz, 2H, C_6H_5), 10.40 (s, 1H, NH). ESI-MS: m/z 324 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{17}N_5O_2$: C, 63.15 H, 5.30; N, 21.66. Found: C, 63.19; H, 5.26; N, 21.54.

4.1.10 N-(4-Bromophenyl)-2-(5-phenyltetrazol-2-yl)acetamide (**9**). Yield 62 %, m.p. 212-213 °C. ^{1}H NMR (400 MHz, DMSO) δ = 5.68 (s, 2H, CH_2), 7.43 (d, *J* = 8.9 Hz, 2H, N-$\underline{C_6H_4}$), 7.49-7.58 (m, 5H, C_6H_5+N-$\underline{C_6H_4}$), 8.11 (dd, *J* = 8.1 Hz, 2H, C_6H_5), 10.72 (s, 1H, NH). ESI-MS: m/z 359 $[M+H]^+$. Anal. Calcd. for $C_{15}H_{12}BrN_5O$: C, 50.30 H, 3.38; N, 22.31. Found: C, 50.41; H, 3.35; N, 22.40.

4.1.11 N-(3-Chloro-2-methylphenyl)-2-(5-phenyltetrazol-2-yl)acetamide (**10**). Yield 69 %, m.p. 151-152 °C. ^{1}H NMR (400 MHz, DMSO) δ = 2.34 (s, 3H, CH_3), 5.74 (s, 2H, CH_2), 7.16 (t, *J* = 8.0, 8.1 Hz, 1H, N-$\underline{C_6H_3}$), 7.24 (d, *J* = 7.9 Hz, 1H, N-$\underline{C_6H_3}$), 7.41 (d, *J* = 7.8 Hz, 1H, N-$\underline{C_6H_3}$), 7.47-7.56 (m, 3H, C_6H_5), 8.11 (d, *J* = 7.5 Hz, 2H, C_6H_5), 10.10 (s, 1H, NH). ESI-MS: m/z 328 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{14}ClN_5O$: C, 58.63 H, 4.31; N, 21.37. Found: C, 58.55; H, 4.37; N, 21.25.

4.1.12 N-(5-Chloro-2-methylphenyl)-2-(5-phenyltetrazol-2-yl)acetamide (**11**). Yield 75 %, m.p. 163-164 °C. ^{1}H NMR (400 MHz, DMSO) δ = 2.30 (s, 3H, CH_3), 5.75 (s, 2H, CH_2), 7.07 (dd, *J* = 8.1 Hz, 1H, N-$\underline{C_6H_3}$), 7.20 (d, *J* = 8.2 Hz, 1H, N-$\underline{C_6H_3}$), 7.49-7.52 (m, 3H, C_6H_5), 7.64 (s, 1H, N-$\underline{C_6H_3}$), 8.11 (dd, *J* = 7.9 Hz, 2H, C_6H_5), 9.93 (s, 1H, NH). ESI-MS: m/z 328 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{14}ClN_5O$: C, 58.63 H, 4.31; N, 21.37. Found: C, 58.69; H, 4.28; N, 21.40.

4.1.13 N-(4-Chloro-3-methylphenyl)-2-(5-phenyltetrazol-2-yl)acetamide (**12**). Yield 75 %, m.p. 182-183 °C. ^{1}H NMR (400 MHz, DMSO) δ = 2.31 (s, 3H, CH_3), 5.67 (s, 2H, CH_2), 7.21 (d, *J* = 8.3 Hz, 1H, N-$\underline{C_6H_3}$), 7.37 (dd, *J* = 8.2 Hz, 1H, N-$\underline{C_6H_3}$), 7.47-7.55 (m, 3H, C_6H_5), 7.73 (s, 1H, N-$\underline{C_6H_3}$), 8.10 (dd, *J* = 7.9 Hz, 2H, C_6H_5), 10.65 (s, 1H, NH). ESI-MS: m/z 328 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{14}ClN_5O$: C, 58.63 H, 4.31; N, 21.37. Found: C, 58.58; H, 4.36; N, 21.44.

4.1.14 N-m-Tolyl-2-(5-p-tolyltetrazol-2-yl)acetamide (**13**). Yield 75 %, m.p. 185-186 °C. ^{1}H NMR (400 MHz, DMSO) δ = 2.32 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 5.64 (s, 2H, CH_2), 6.87 (d, *J* = 7.4 Hz 1H, N-$\underline{C_6H_4}$), 7.16 (t, *J* = 7.7, 8.0 Hz, 1H, N-$\underline{C_6H_4}$), 7.31-7.37 (m, 3H, C_6H_5+N-$\underline{C_6H_4}$), 7.42 (s, 1H, N-$\underline{C_6H_4}$), 7.98 (d, *J* = 8.1 Hz, 2H, C_6H_5), 10.48 (s, 1H, NH). ESI-MS: m/z 308 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{17}N_5O$: C, 66.43 H, 5.58; N, 22.79. Found: C, 66.36; H, 5.51; N, 22.64.

4.1.15 N-(4-Chlorophenyl)-2-(5-p-tolyltetrazol-2-yl)acetamide (**14**). Yield 84 %, m.p. 186-187 °C. ^{1}H NMR (400 MHz, DMSO) δ = 2.32 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 5.67 (s, 2H, CH_2), 7.28-7.33 (m, 4H, C_6H_5+ N-$\underline{C_6H_4}$), 7.62 (d, *J* = 8.8 Hz, 2H, N-$\underline{C_6H_4}$), 7.97 (d, *J* = 8.0 Hz, 2H, C_6H_5), 10.81 (s, 1H, NH). ESI-MS: m/z 328 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{14}ClN_5O$: C, 58.63 H, 4.31; N, 21.37. Found: C, 58.57; H, 4.35; N, 21.44.

4.1.16 N-(4-Ethoxyphenyl)-2-(5-p-tolyltetrazol-2-yl)acetamide (**15**). Yield 77 %, m.p. 177-178 °C. ^{1}H NMR (400 MHz, DMSO) δ = 1.36 (t, *J* = 7.0 Hz, 3H, O-$CH_2\underline{CH_3}$), 2.42 (s, 3H, CH_3), 3.96-4.07 (m, 2H, O-$\underline{CH_2}CH_3$), 5.61 (s, 2H, CH_2), 6.81 (d, *J* = 8.8 Hz, 2H, N-$\underline{C_6H_4}$), 7.32 (d, *J* = 8.0 Hz, 2H, C_6H_5), 7.48 (d, *J* = 8.8 Hz, 2H, N-$\underline{C_6H_4}$), 7.98 (d, *J* = 8.0 Hz, 2H, C_6H_5), 10.41 (s, 1H, NH). ESI-MS: m/z 338 $[M+H]^+$. Anal. Calcd. for $C_{18}H_{19}N_5O_2$: C, 64.08 H, 5.68; N, 20.76. Found: C, 63.96; H, 5.74; N, 20.81.

4.2 Pharmacological/biological assays

4.2.1 Anticancer activity

The tested substances was added to the culture at a alone concentration (10^{-5} M), and the cultures were incubated for 48 h. Endpoint definition was carried out with a protein-binding dye, sulforhodamine B (SRB). Results for every tested compound were reported as the percent growth of the processed cells when compared to the untreated control cells (http://dtp.nci.nih.gov). The percent growth was evaluated spectrophotometrically versus not processed controls. The cytotoxic and/or growth inhibitory effects of the most active substancess were tested in vitro contrary the full panel of about 60 human cancer cell lines at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. The 48-h continuous drug exposure protocol was followed, and an SRB protein assay was used to estimate cell viability or growth. Using the seven absorbance measurements [time zero, (Tz), control growth in the lack of drug, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

$[(Ti - Tz)/(C - Tz)] \times 100$ for concentrations for which $Ti \geq Tz$;
$[(Ti - Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$.

Three dose-response parameters were calculated for every compound. Growth inhibition of 50% (GI50) was calculated of [(Ti − Tz)/(C − Tz)] × 100 − 50, which is the drug concentration resulting in a 50% below net protein magnification in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50% contraction in the measured protein at the end of the drug treatment as compared to that at the starting) indicating a net loss of cells next treatment was calculated from [(Ti − Tz)/Tz] × 100 = -50. Significance were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was pronounced as more or less than the maximum or minimum concentration was tested.

4.2.2 Antimicrobial activity

The study of the antifungal and antibacterial action of the synthesized compounds was carried out using the micromethod of two-fold serial dilutions in a liquid nutrient medium. The minimum bacteriostatic or fungistatic MIC – MBsC, MFsC) concentrations and the minimum bactericidal or fungicidal (MBcC, MFcC) concentrations of the synthesized compounds were determined relative to the fungicidal strain *C. albicans ATCC 885/653* and the reference bacterial strains *S. aureus ATCC 25923* and *E. coli ATCC 25922*.

Up to 96 well polystyrene plates were added 0.05 ml of a 4-hour culture of microorganisms. For fungicidal, 10^4 CFU/ml were used in Sabouraud's liquid medium. For bacteria, 1 ml of mesopatamia broth contained 10^5 CFU/ml. A suspension of the studied microorganisms (inoculum) was prepared from a daily culture. Several isolated colonies of the same type were selected with a loop for inoculation. Then a small amount of material was transferred into a tube with sterile saline solution. Using a densitometer (DEN-1 Biosan), a suspension of microorganisms was obtained at a concentration of 1.5×10^8 CFU/ml. The resulting concentration corresponded to a McFarland 0.5 turbidity standard. After 15 min, the necessary working microbial suspension was obtained by tenfold dilution in a nutrient medium. Solutions of test compounds were prepared for the micromethod of serial dilutions at a concentration of 1000 μg/ml. DMSO was used as a solvent. The basic working solutions were stored at a temperature not exceeding 20 °C. In the first well was filled with 0.05 ml of the matrix solution of the research substance. After stirring, on 0.05 ml was transferred into the subsequent wells of the first row. In this way, dilutions from 500 μg/ml to 3.9 μg/ml were obtained. Studies were also carried out in the following rows of holes with other compounds. After that, the plates were placed in a thermostat. For bacteria at 37° C, incubated for 24 hours. For fungi, at 28° C, incubated for 48 hours. The minimum concentration of the test substance, in the presence of which no culture growth was observed, was taken as the bacteriostatic (fungistatic) concentration. The experiments were carried out in parallel with the control. The experiments were carried out in parallel three times to obtain reliable results. Using each concentration of the compound and the studied culture of microorganisms.

Bactericidal (fungicidal) concentrations of the test compounds were determined by removing the reference strain of the microorganism from the medium with the test compound and then reseeding on a solid nutrient medium without the test compound. Absence of growth from wells with a certain concentration of test compound after reseeding was defined as the acid concentration of this compound.

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