Novel pyrazole bearing heterocyclic hybrids as promising biologically active compounds

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The paper presents the results of a study devoted to the synthesis, evaluation of physicochemical properties, antiradical, and antimicrobial activity of pyrazole-containing heterocyclic hybrids. It has been shown that pyrazole bearing heterocyclic hybrids that contain quinazoline, triazoloquinazoline or triazole moieties are available via a series of reactions that include the interaction of 4-chloroquinazoline with pyrazole-containing hydrazides, cyclization of formed products into corresponding triazoloquinazolines, and hydrolytic cleavage of the electron-deficient tricyclic system. The structures of synthesized compounds have been verified by a complex of physicochemical methods, and the features of ^{1}H NMR spectral characteristics have been described as well. The evaluation of obtained compounds' biological activity revealed a moderate bacteriostatic effect against Pseudomonas aeruginosa and Candida albicans, as well as high radical scavenging activity of some of the studied heterocyclic hybrids.

Key words: heterocyclic hybrids, pyrazole, quinazoline, triazole, antiradical activity.

Nové pyrazolové heterocyklické hybridy jako slibné biologicky aktivní sloučeniny

Tento článek představuje výsledky studie zaměřené na syntézu, hodnocení fyzikálně-chemických vlastností, antiradikálové a antimikrobiální aktivity pyrazolových heterocyklických hybridů. Bylo prokázáno, že heterocyklické hybridy nesoucí pyrazol, které obsahují chinazolin, triazolochinazolin nebo triazolové skupiny, jsou dostupné prostřednictvím série reakcí zahrnujících interakci 4-chlorochinazolinu s pyrazolovými hydrazidy, cyklizaci vytvořených produktů na odpovídající triazolochinazolinové deriváty a hydrolytický rozklad elektronově deficitního tricyklického systému. Struktury syntetizovaných sloučenin byly ověřeny komplexem fyzikálně-chemických metod a byly popsány i charakteristické znaky ^{1}H NMR spektrálních vlastností. Hodnocení biologické aktivity získaných sloučenin odhalilo mírný bakteriostatický účinek proti Pseudomonas aeruginosa a Candida albicans, stejně jako vysokou schopnost některých studovaných heterocyklických hybridů zachytávat radikály.

Klíčová slova: heterocyklické hybridy, pyrazol, chinazolin, triazol, antiradikálová aktivita.

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Introduction

Pyrazoles have remained one of the most studied classes of heterocyclic compounds over the last few decades due to their synthetic availability, wide possibilities of chemical modification, and valuable properties (1). Particularly promising in the scope of purposeful search for novel bioactive molecules are studies aimed at the synthesis of heterocyclic hybrids containing pyrazole moieties. Among recently published papers, a study devoted to the synthesis and biological evaluation of molecular hybrids containing benzimidazole and pyrazole motifs is noteworthy (2). It has been shown that 2-(1H-benzo[d]imidazol-2-yl)-3-(1,3-diaryl-1H-pyrazol-4-yl)acrylonitriles (Figure 1, A) reveal radical scavenging and anti-inflammatory activity. Also, the studied compounds demonstrated growth inhibiting activity against human pancreatic cancer cells. Anti-inflammatory activity is also observed for pyrazole-coumarin hybrids (Figure 1, B), which also exhibit antimicrobial properties (3). Kuthyala et al. described a series of substituted bipyrazole-imidazole and bipyrazole-dihydropyrimidine molecular hybrids (Figure 1, C, D) possessing anticancer activity (4). Heterocyclic hybrids bearing a pyrazole moiety have been repeatedly noted as antimicrobial agents (5-7). Thus, 4-(4,5-dihydro-1H-imidazol-2-yl)-1-aryl-1H-pyrazol-5-amines and 1-aryl-4-(1,4,5,6-tetrahydropyrimidin-2-yl)--1H-pyrazol-5-amines (Figure 1, E) have been identified as promising trypanocide compounds (5), pyrazole-oxadiazole hybrids (Figure 1, F) as antitubercular agents (6), and compounds combining pyrazole with benzoxazole or benzothiazole fragments (Figure 1, G) as fungicides (7).

Significant antimicrobial activity has been described for complex hybrids (Figure 1, H) combining pyrazole, benzothiazole, thiazole, and coumarin fragments (8). The search for novel α-amylase and α-glycosidase inhibitors among pyrazole-containing heterocyclic hybrids is another promising trend in medicinal chemistry. The abovementioned activities have been described for thiazolidine-pyrazole (9) (Figure 1, I) and pyrazole-triazolopyrimidine hybrids (10) (Figure 1, J). It can be concluded that pyrazole-containing heterocyclic hybrids are the focus of studies aimed at the elaboration of novel bioactive agents; however, the potential of this direction has not been exhausted. Moreover, a significant part of target molecular hybrids can be obtained only using multistep procedures characterized by low yields.

Considering the above, the present study is devoted to the elaboration of simple procedures for the preparation of pyrazole – quinazoline, pyrazole-triazoloquinazoline and pyrazole-triazole hybrids (Figure 2) and the evaluation of their radical scavenging and antimicrobial activities.

Experimental

Materials and Methods

The Stuart SMP30 apparatus (Stuart Equipment, UK) was used for estimating melting point values. ^{1}H NMR spectra were recorded on a Varian-Mercury 400 spectrometer (Varian Inc., US) using hexadeuterated dimethylsulfoxide as a solvent and tetramethylsilane as an internal standard. LC-MS data were registered on an HPLC-MS system consisting of an Agilent 1100 Series chromatograph and a diode-matrix/mass-selective detector Agilent LC/MSD SL (APCI) (Agilent Technologies,

Figure 2. *The structure of target compounds*

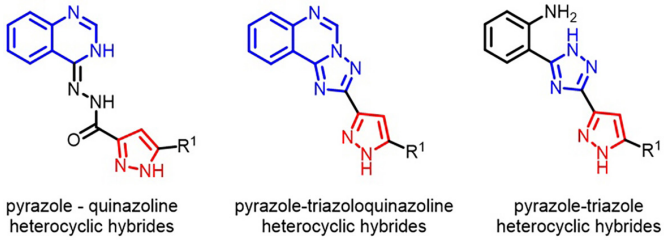
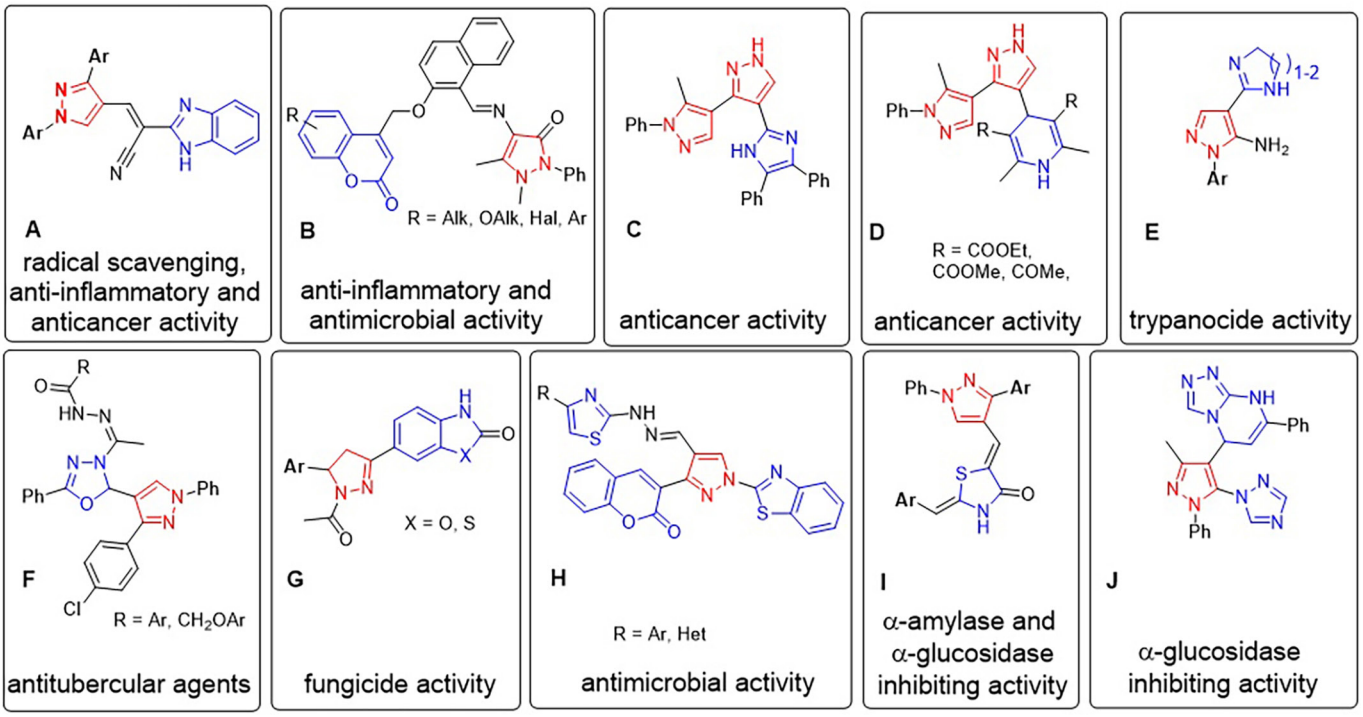


Figure 1. *Pyrazole bearing heterocyclic hybrids with significant biological activity*



Inc., US). ANG200C electronic scales (Axis, Gdansk, Poland) were used for weighing the studied compounds and reagents. Optical density was measured using a ULAB 108UV spectrophotometer (Ulab, Shanghai, China).

5-R1-pyrazole-3-carbohydrazides were obtained according to described method (11). All other reagents and solvents were purchased from Enamine Ltd (Ukraine) and used without additional purification.

Synthetic procedure for 5-R^1-N′-(quinazolin-4(3H)-ylidene)-1H-pyrazole-3-carbohydrazides (2.1-2.9): 20 mmol (3.28 g) of 4-chloroquinazoline was dissolved in 30 ml of dioxane under heating. The formed solution was cooled to room temperature, and 20 mmol of triethylamine and 20 mmol of the corresponding 5-R^1-pyrazole-3-carbohydrazide were added, along with 50 ml of dioxane. The formed reaction mixture was refluxed for 3 hours. The reaction mixture was then cooled and poured into 600 ml of water. The formed precipitate was filtered off, washed with water, and dried. Recrystallization from a methanol-water mixture (1:1) was used for additional purification.

5-methyl-N′-(quinazolin-4(3H)-ylidene)-1H-pyrazole-3-carbohydrazide 2. 1. Yield: 80%; gray crystalline powder; M.p. 244-246°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 12.75 (s, 1H, NH-pyrazole), 11.03 (s, 1H, quinazoline 3-NH), 10.89 (s, 1H, C(O)NNH), 8.66 (s, 1H, quinazoline H-2), 8.54 (d, J = 7.6 Hz, 1H, quinazoline H-5), 7.98 – 7.80 (m, 2H, quinazoline H-7,8), 7.64 (t, 1H, quinazoline H-6), 6.47 (s, 1H, pyrazole H-4), 2.34 (s, 3H, CH3); LC-MS: purity 100%, m/z = 269.

5-phenyl-N′-(quinazolin-4(3H)-ylidene)-1H-pyrazole-3-carbohydrazide 2. 2. Yield: 86%; white crystalline powder; M.p. 256-258°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 13.66 (s, 1H, NH-pyrazole), 11.08 (s, 1H, NHCO), 8.20 (m, 2H, quinazoline H-2,5), 7.79 (d, J = 7.4 Hz, 2H, Ar H-2,6), 7.61 – 7.21 (m, 6H, quinazoline H-6,7,8, Ar H-3,4,5), 7.10 (s, 1H, pyrazole H-4); LC-MS: purity >95%, m/z = 331.

5-(2-bromophenyl)-N′-(quinazolin-4(3H)-ylidene)-1H-pyrazole-3-carbohydrazide 2. 3. Yield: 85%; yellow crystalline powder; M.p. 244-245°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 13.69 (s, 1H, NH-pyrazole), 11.15 (s, 1H, NHCO), 8.96 – 8.21 (m, 2H, quinazoline H-2,5), 7.87 – 7.54 (m, 5H, quinazoline H-6, 8, Ar H-2,3,6), 7.46 (t, J = 7.2 Hz, 1H, quinazoline H-6), 7.37 – 7.26 (m, 1H, Ar H-5), 7.17 (s, 1H, pyrazole H-4); LC-MS: purity 100%, m/z = 409.

5-(4-nitrophenyl)-N′-(quinazolin-4(3H)-ylidene)-1H-pyrazole-3-carbohydrazide 2. 4. Yield: 71%; orange crystalline powder; M.p. 270-271oC; 1H NMR (400 MHz, DMSO-d6) δ 14.10 (s, 1H, NH-pyrazole), 11.12 (s, 1H, NHCO), 8.39 – 8.17 (m, 4H, quinazoline 2,5, Ar H-3,5), 8.12 (d, J = 8.9 Hz, 2H, Ar H-2,6), 7.84 – 7.25 (m, 4H, quinazoline H-6,7,8, pyrazole H-4); LC-MS: purity >95%, m/z = 376.

5-(4-cyanophenyl)-N′-(quinazolin-4(3H)-ylidene)-1H-pyrazole-3-carbohydrazide 2. 5. Yield: 91%; orange crystalline powder; M.p. 229-230°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 13.99 (s, 1H, NH-pyrazole), 11.11 (s, 1H, NHCO), 8.22 (s, 1H, quinazoline H-2), 8.02 (d, J = 7.5 Hz, 2H, Ar H-3,5), 7.81 (d, J = 7.3 Hz, 2H, Ar H-2,6), 7.62 (m, 1H, quinazoline H-5), 7.54 – 7.00 (m, 4H, quinazoline H-6,7,8, pyrazole H-4); LC-MS: purity 100%, m/z = 356.

5-(4-methoxyphenyl)-N′-(quinazolin-4(3H)-ylidene)-1H-pyrazole-3-carbohydrazide 2. 6. Yield: 95%; pale pink crystalline powder; M.p. 208-209°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 13.46 (s, 1H, NH-pyrazole), 11.05 (s, 1H, NHCO), 8.17 – 7.98 (m, 2H, quinazoline H-2,5), 7.70 (d, J = 7.6 Hz, 2H, Ar H-2,6), 7.50 (t, 1H, quinazoline H-7), 7.40 – 7.21 (m, 2H, quinazoline H-6,8), 7.07 – 6.87 (m, 3H, pyrazole H-4, Ar H-3,5), 3.82 (s, 3H, OCH3); LC-MS: purity 100%, m/z = 361.

5-(2,4-difluorophenyl)-N′-(quinazolin-4(3H)-ylidene)-1H-pyrazole-3-carbohydrazide 2. 7. Yield: 91%; white crystalline powder; M.p. 220-221°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 13.69 (s, 1H, NH-pyrazole), 11.09 (s, 1H, NHCO), 8.27 – 8.08 (m, 2H, quinazoline 2,5), 7.99 – 7.83 (m, 1H, Ar H-3), 7.63 – 7.48 (m, 1H, Ar H-5), 7.47 – 7.27 (m, 2H, quinazoline H-6, 8), 7.17 – 6.88 (m, 3H, quinazoline H-7, Ar H-6, pyrazole H-4); LC-MS: purity 100%, m/z = 367.

N′-(quinazolin-4(3H)-ylidene)-5-(3,4,5-trimethoxyphenyl)-1H-pyrazole-3-carbohydrazide 2. 8. Yield: 91%; pale pink crystalline powder; M.p. 198-199°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 13.62 (s, 1H, NH-pyrazole), 11.04 (s, 1H, NHCO), 8.39 – 8.16 (m, 2H, quinazoline H-2,5), 7.65 (t, 1H, quinazoline H-7), 7.56 – 7.37 (m, 2H, quinazoline H-6,8), 7.18 (s, 1H, pyrazole H-4), 7.11 (s, 2H, Ar H-2,6), 3.89 (s, 6H, 3,5-(OCH3)2), 3.72 (s, 3H, 4-OCH3); LC-MS: purity 100%, m/z = 421.

5-(furan-2-yl)-N′-(quinazolin-4(3H)-ylidene)-1H-pyrazole-3-carbohydrazide 2. 9. Yield: 90%; pale pink crystalline powder; M.p. 245-246°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 13.66 (s, 1H, NH-pyrazole), 11.06 (s, 1H, NHCO), 8.18 – 7.94 (m, 2H, furan H-5, quinazoline H-5), 7.64 (s, 1H, quinazoline H-2), 7.49 (d, 1H, quinazoline H-8), 7.40 – 7.18 (m, 2H, quinazoline H-6,7), 6.91 (d, 1H, furan H-3), 6.84 (t, 1H, furan H-4), 6.54 (s, 1H, pyrazole H-4); LC-MS: purity 100%, m/z = 321.

Synthetic procedure for 2-(5-R1-1H-pyrazol-3-yl)-[1,2,4]triazolo[1,5-c]quinazolines(3.1-3.8). The solution of 20 mmol of corresponding 5-R1-N′-(quinazolin-4(3H)-ylidene)-1H-pyrazole-3-carbohydrazide in 50 ml of acetic acid was refluxed for 5 hours with separation of the formed water. After completing of reaction, the mixture was cooled and the formed precipitate was filtered off, washed by methanol and dried. Recrystallization from a propanol-2 was used for additional purification.

2-(5-methyl-1H-pyrazol-3-yl)-[1,2,4]triazolo[1,5-c]quinazoline 3. 1. Yield: 82%; white crystalline powder; M.p. 280-282°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.45 (s, 1H, H-5), 8.40 (d, J = 8.2 Hz, 1H, H-10), 7.95 (t, J = 7.3 Hz, 1H, H-8), 7.82 (d, J = 7.4 Hz, 1H, H-7), 7.69 (t, J = 7.5 Hz, 1H, H-9), 7.09 (s, 1H, pyrazole H-4), 2.40 (s, 3H, CH3); purity >95%, LC-MS: m/z = 251.

2-(5-phenyl-1H-pyrazol-3-yl)-[1,2,4]triazolo[1,5-c]quinazoline 3. 2. Yield: 89%; white crystalline powder; M.p. 295-296°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.65 (s, 1H, H-5), 8.50 (d, J = 8.7 Hz, 1H, H-10), 8.02 (t, J = 6.3 Hz, H-8), 7.94 (d, 1H, H-7), 7.82 (t, J = 7.6 Hz, 1H, H-9), 7.72 (d, J = 8.0 Hz, Ar H-2,6), 7.53-7.42 (m, 3H, Ar H-3,4,5), 7.20 (s, 1H, pyrazole H-4); LC-MS: purity >95%, m/z = 313.

2-(5-(2-bromophenyl)-1H-pyrazol-3-yl)-[1,2,4]triazolo[1,5-c]quinazoline 3. 3. Yield: 85%; pale yellow crystalline powder; M.p. 287-288°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 11.18 (s, 1H, pyrazole H-1), 9.56 (d, J = 2.6 Hz, 1H, H-5), 8.57 (d, J = 7.6 Hz, 1H, H-10), 8.08 (d, J = 8.0 Hz, 1H, H-7), 7.93 (t, J = 7.5 Hz, 1H, H-8), 7.83 (t, J = 7.4 Hz, 1H, H-9), 7.79 – 7.62 (m, 2H, Ar H-3,6), 7.46 (t, 1H, Ar H-4), 7.38 (s, 1H, pyrazole H-4), 7.30 (t, 1H, J = 7.5 Hz, Ar H-5); LC-MS: purity >95%, m/z = 391.

2-(5-(4-nitrophenyl)-1H-pyrazol-3-yl)-[1,2,4]triazolo[1,5-c]quinazoline 3. 4. Yield: 75%; brown crystalline powder; M.p. 301-302°C; ^{1}H NMR

(400 MHz, DMSO-d6) δ 9.92 (s, 1H, H-5), 8.40 – 8.18 (m, 4H, H-8,10, Ar H-3,5), 8.13 – 7.99 (m, 3H, H-7, Ar H-2,6), 7.48 (s, 1H, H-9), 7.41 (s, 1H, pyrazole H-4); purity 100%, LC-MS: m/z = 358.

4-(3-([1,2,4]triazolo[1,5-c]quinazolin-2-yl)-1H-pyrazol-5-yl)benzonitrile 3. 5. Yield: 82%; white crystalline powder; M.p. 290-291°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.72 (s, 1H, H-5), 8.45 (d, 1H, H-10), 8.18-8.02 (m, 3H, H-7, Ar H-3,5), 7.95 (t, 1H, H-8), 7.89-7.69 (m, 3H, H-9, Ar H-2,6), 7.38 (s, 1H, pyrazole H-4); LC-MS: purity >95%, m/z = 338.

2-(5-(4-methoxyphenyl)-1H-pyrazol-3-yl)-[1,2,4]triazolo[1,5-c]quinazoline 3. 6. Yield: 82%; pale pink crystalline powder; M.p. 248-249°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.54 (s, 1H, H-5), 8.54 (d, J = 7.6 Hz, 1H, H-10), 8.07 (d, J = 7.9 Hz, 1H, H-7), 7.91 (t, J = 7.8 Hz, 1H, H-8), 7.87 – 7.72 (m, 3H, H-9, Ar H-2,6), 7.19 (s, 1H, pyrazole H-4), 6.97 (d, J = 7.8 Hz, 2H, Ar H-3,5), 3.83 (s, 3H, OCH3); LC-MS: purity >95%, m/z = 343.

2-(5-(2,4-difluorophenyl)-1H-pyrazol-3-yl)-[1,2,4]triazolo[1,5-c]quinazoline 3. 7. Yield: 78%; white crystalline powder; M.p. 295-296°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.56 (s, 1H, H-5), 8.56 (d, J = 8.9 Hz, 1H, H-10), 8.07 (t, J = 7.0 Hz, 1H, H-8), 7.92 (d, J = 7.1 Hz, 1H, H-7), 7.84 (t, J = 7.2 Hz, 1H, H-9), 7.29 (s, 1H, pyrazole H-4), 7.22 – 7.04 (m, 3H, Ar H-3,5,6); LC-MS: purity 100%, m/z = 349.

2-(5-(furan-2-yl)-1H-pyrazol-3-yl)-[1,2,4]triazolo[1,5-c]quinazoline 3. 8. Yield: 90%; yellow crystalline powder; M.p. 285-286°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.53 (s, 1H, H-5), 8.52 (d, J = 7.8 Hz, 1H, H-10), 8.05 (d, J = 8.1 Hz, 1H, H-7), 7.90 (t, J = 7.6 Hz, 1H, H-8), 7.81 (t, J = 7.5 Hz, 1H, H-9), 7.62 (d, 1H, furan H-5), 7.12 (s, 1H, pyrazole H-4), 6.87 – 6.73 (m, 1H, furan H-3), 6.61 – 6.45 (m, 1H, furan H-4); purity >95%, LC-MS: m/z = 303.

Synthetic procedure for 2-(3-(5-R1-1H-pyrazol-3-yl)-1H-1,2,4-triazol-5-yl)anilines (4.1-4.6): The suspension of 10 mmol of the corresponding 2-(5-R1-1H-pyrazol-3-yl)-[1,2,4]triazolo[1,5-c]quinazoline in 50 ml of a 10% aqueous solution of hydrochloric acid was refluxed for 6 hours. After completion of the reaction, the mixture was cooled, poured into a solution of sodium acetate, and the formed precipitate was filtered off, washed with water, and dried. Recrystallization from a methanol-water mixture was used for additional purification.

2-(3-(5-methyl-1H-pyrazol-3-yl)-1H-1,2,4-triazol-5-yl)aniline 4. 1. Yield: 80%; white crystalline powder; M.p. 208-209oC; 1H NMR (400 MHz, DMSO-d6) δ 8.46 (s, 2H, NH2), 8.24 (d, J = 7.7 Hz, 1H, H-3), 7.48 (d, J = 7.9 Hz, 1H, H-6), 7.43 (t, J = 7.9 Hz, 1H, H-5), 7.29 (t, J = 6.8 Hz, 1H, H-4), 6.84 (s, 1H, pyrazole H-4), 2.40 (s, 3H, CH3); purity >95%, LC-MS: m/z = 241.

2-(3-(5-phenyl-1H-pyrazol-3-yl)-1H-1,2,4-triazol-5-yl)aniline 4. 2. Yield: 87%; white crystalline powder; M.p. 201-202°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 13.97 – 13.22 (m, 2H, pyrazole-NH, triazole-NH), 7.97 – 7.71 (m, 3H, H-3, Ar H-2,6), 7.41 (t, J = 7.4 Hz, 2H, Ar H-3,5), 7.30 (t, J = 6.8 Hz, 1H, H-5), 7.12 (s, 1H, pyrazole H-4), 7.08 (t, J = 7.5 Hz, 1H, H-4), 6.79 (d, J = 8.0 Hz, 1H, H-6), 6.71 – 6.40 (m, 3H, NH2, Ar H-4); LC-MS: purity 100%, m/z = 303.

2-(3-(5-(2-bromophenyl)-1H-pyrazol-3-yl)-1H-1,2,4-triazol-5-yl)aniline 4. 3. Yield: 75%; pale pink crystalline powder; M.p. 208-209°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 14.95 – 12.63 (m, 2H, pyrazole-NH, triazole-NH), 8.02 – 7.86 (m, 1H, H-3), 7.74 (d, J = 6.8 Hz, 1H, Ar H-6), 7.69 (d, J = 7.8 Hz, 1H, Ar H-3), 7.43 (t, J = 7.2 Hz, 1H, Ar H-4), 7.28 (t, 2H, Ar H-5), 7.20 (s, 1H, pyrazole H-4), 7.15 (t, 1H, H-4), 6.91 (d, J = 7.2 Hz, 1H, H-6), 6.71 (s, 2H, NH2); LC-MS: purity >95%, m/z = 381.

4-(3-(5-(2-aminophenyl)-1H-1,2,4-triazol-3-yl)-1H-pyrazol-5-yl)benzonitrile 4. 4. Yield: 77%; yellow crystalline powder; M.p. 243-244°C;^{1}H NMR (400 MHz, DMSO-d6) δ 14.06 (m, 2H, triazine-NH, pyrazole-NH), 8.20 – 8.02 (m, 3H, H-3, Ar H-3,5), 7.89 – 7.66 (d, 2H, Ar H-2,6), 7.26 (s, 1H, pyrazole H-4), 7.10 (t, J = 6.3 Hz, 1H, H-5), 6.91 – 6.73 (m, 1H, H-4), 6.68 – 6.49 (m, 1H, H-6); LC-MS: purity >95%, m/z = 328.

2-(3-(5-(4-methoxyphenyl)-1H-pyrazol-3-yl)-1H-1,2,4-triazol-5-yl) aniline 4. 5. Yield: 87%; pale pink crystalline powder; M.p. 222-223°C; ^{1}H NMR (400 MHz, DMSO-d6) 8.04 (d, J = 5.3 Hz, 1H, H-3), 7.77 (d, 2H, Ar-2,6), 7.24 (t, J = 7.3 Hz, 1H, H-5), 7.14 – 7.04 (m, 2H, pyrazole H-4, H-6), 7.04 – 6.75 (m, 3H, Ar H-3,5, H-4), 3.83 (s, 3H, OCH3); LC-MS: purity 100%, m/z = 333.

2-(3-(5-(furan-2-yl)-1H-pyrazol-3-yl)-1H-1,2,4-triazol-5-yl)aniline 4. 6. Yield: 87%; yellow crystalline powder; M.p. 212-213°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 14.35 – 13.81 (m, 2H, pyrazole NH, triazole NH), 7.90 – 7.82 (m, 1H, H-3), 7.60 (d, 1H, furan H-5), 7.11 (t, J = 7.1 Hz, 1H, H-5), 6.97 (d, 1H, furan H-3), 6.83 (d, J = 8.0 Hz, 1H, H-6), 6.79 – 6.74 (m, 1H, H-4), 6.63 (t, 1H, furan H-4), 6.52 (s, 1H, pyrazole H-4); LC-MS: purity 100%, m/z = 293.

Biological studies

Radical scavenging activity

A 2 ml solution of the studied substances in DMSO at concentrations of 2 mM or 0.2 mM was mixed with 2 ml of a 0.1 mM methanol solution of 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazin-1-yl (DPPH). The formed mixture was incubated for 30 min at ambient temperature, and its absorption was measured (AD). The optical density of the mixture containing 2 ml of a 0.1 mM methanol solution of DPPH and 2 ml of DMSO (ADPPH) was measured as well (12). Antiradical activity (ARA%) was calculated using the formula:

$$ARA\% = \frac{ADPPH - AD}{ADPPH} \times 100\%$$

Antimicrobial test

Sensitivity of microorganisms to synthesized compounds were evaluated according described methods (13). Assay was conducted on Mueller-Hinton medium by two-fold serial dilution of compound in 1 ml, after that 0.1 ml of microbial seeding (10^6 cells/ml) was added. Minimal inhibit concentration of compound was determined by absence of visual growth in test tube with minimal concentration of substance, minimal bactericide/fungicide concentration was determined by absence of growth on agar after inoculation of microorganism from transparent test-tubes. Dimethylsulfoxide was used as a solvent, initial solution concentration was 1 mg/ml Preliminary screening was performed on 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 Zaporizhzhia Regional Laboratory Center of State Sanitary and Epidemiological Service of Ukraine. Nitrofual and Trimetoprim were used as reference compound with proved antibacterial/antifungal activity.

Results and Discussion

The synthesis of the pyrazole–quinazoline hybrids 2 was conducted using the previously described procedure (14), namely via the interaction between 4-chloroquinazoline and 5-R[1]-1H-pyrazole-3-carbohydrazides in the presence of triethylamine (Figure 3). The annelation of the triazole moiety to the quinazoline system was performed by refluxing compounds 2 in glacial acetic acid with the separation of the formed water (14). The abovementioned approach allowed obtaining target triazoloquinazoline–pyrazole hybrids (compounds 3) with high yields (Figure 3). The formation of compounds 3 proceeds as cyclization followed by ANRORC-isomerization of unstable intermediate [1,2,4]triazolo[4,3-c]quinazolines (14). It should be mentioned that the triazoloquinazoline system is highly electron-deficient and consequently able to undergo hydrolytic cleavage under the action of aqueous solutions of mineral acid. It has been shown that refluxing compounds 3 in a 10% aqueous solution of hydrochloric acid for 6 hours results in the degradation of the pyrimidine cycle and the formation of 2-(3-(5-R[1]-1H-pyrazol-3-yl)-1H-1,2,4-triazol-5-yl) anilines (compounds 4) (Figure 3) (15). ^{1}H NMR and LC/MS methods were used for the estimation of the purity and structure of obtained compounds.

Synthesized compounds were evaluated for their antiradical and antimicrobial activities (Table 1). Radical scavenging activity of synthesized compounds was studied to evaluate their possible pharmacological potential. It has been found that 5-R[1]-N′-(quinazolin-4(3H)-ylidene)-1H-pyrazole-3-carbohydrazides (compounds 2) at

Figure 3. *Synthesis of pyrazole bearing heterocyclic hybrids*

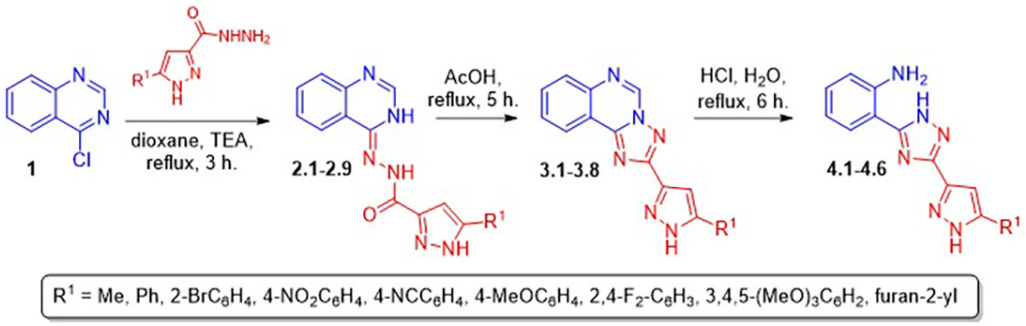


Table 1. *Antiradical activity of synthesized compounds*

Comp.	ARA%,1 mM	ARA%,0.1 mM	Comp.	ARA%,1 mM	ARA%,0.1 mM
Ascorbic acid	95	82	3.6	0	0
2.3	89	56	3.7	51	4
2.5	91	63	3.8	34	5
2.6	93	68	4.3	89	24
2.7	93	64	4.4	79	11
2.8	83	51	4.5	87	45
2.9	84	60	4.6	79	9
3.4	14	9	—	—	—

Table 2. *Antibacterial activity of synthesized compounds*

Compound	E. coli		S. aureus		P. aerugin.		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	100	200	100	200	100	200	50	100
2.5	100	200	50	100	50	100	50	50
2.6	100	200	50	100	200	200	50	50
2.8	100	200	100	200	100	100	50	50
2.9	100	200	100	200	100	200	50	100
3.3	100	200	100	200	50	100	100	100
3.4	100	200	100	200	50	200	50	100
3.6	100	200	100	200	50	100	50	100
3.7	100	200	100	200	100	200	50	100
3.8	100	200	100	200	100	200	50	100
4.1	100	200	100	200	100	100	50	100
4.2	100	200	100	200	100	200	50	100
4.3	100	200	100	200	50	100	100	100
4.4	100	200	100	200	50	200	100	100
4.5	100	200	100	200	50	100	50	100
4.6	100	200	100	200	50	100	50	100
Trimetoprim	50	50	31.2	62.5	62.5	125	62.5	125
Nitrofural	1.5	—	6.25	—	6.25	—	25.0	—

a concentration of 1 mM reveal high antiradical (84.08-92.8%) activity comparable with the effect of the reference compound (ascorbic acid). It should be mentioned that radical scavenging activity of compounds 2 is preserved (51.05-68.15%) at a concentration of 0.1 mM (Table 1). The formation of the tricyclic system (compounds 3) keenly decreases antiradical activity (50.6-0% at a concentration of 1 mM, 8.6-0% at a concentration of 0.1 mM) (Table 1).

The study of synthesized compounds' antimicrobial activity was conducted against Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, and Escherichia coli microorganisms (Table 2). It has been estimated that obtained compounds are moderately or low active against studied microorganisms. According to experimental data, Escherichia coli and Staphylococcus aureus were not sensitive towards obtained compounds (MIC and MBC ≥100 μg/ml).

Compounds 2.5, 3.3, 3.4, 3.6, 4.3, 4.4, 4.5, and 4.6 reveal slight bacteriostatic activity against Pseudomonas aeruginosa (MIC = 50 μg/ml). Candida albicans was found to be the most sensitive microorganism towards obtained compounds. Thus, most of the studied compounds reveal fungistatic activity against Candida albicans at a dose of 50 μg/ml (Table 2).

Conclusions

It has been shown that a series of reactions starting from 4-chloroquinazoline and 5-R^1-1H-pyrazole-3-carbohydrazides allowed obtaining heterocyclic hybrids that combine in their structure a pyrazole fragment with quinazoline, triazinoquinazoline, or triazole moieties. Obtained compounds were found to be not active against Escherichia coli and Staphylococcus aureus. At the same time, some of the studied substances at a concentration of 50 μg/ml reveal bacteriostatic effect against Pseudomonas aeruginosa and fungistatic activity against Candida albicans. 5-R^1-N'-(quinazolin-4(3H)-ylidene)-1H-pyrazole-3-carbohydrazides were the only among synthesized classes of compounds which revealed radical-scavenging properties comparable with the activity of the reference compound ascorbic acid. Hence, the abovementioned substances are interesting as objects for the studies aimed at the evaluation of the activities associated with radical scavenging mechanisms (anti-ischemic, antioxidant, anti-inflammatory, etc.).

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