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SYNTHESIS AND MODIFICATION OF 2-[8-R_1-9-R_2-10-R_3-3-R-2-OXO-2*H*-[1,2,4]TRIAZINO[2,3-*c*]QUINAZOLINE-6-YL) THIO]ACETIC ACIDS AIMED AT SEARCHING EFFECTIVE SUBSTANCES WITH THE ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

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In the present paper 50 new derivatives of 2-[8-R_1-9-R_2-10-R_3-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c] quinazoline-6-yl)thio]acetic acids have been described. It has been shown that alkylation of potassium 8-R_1-9-R_2-10-R_3-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-thiolates by chloracetic acid, chloracetamide, N-R_4-chloracetamides and chloracetonitrile yield the corresponding 2-[8-R_1-9-R_2-10-R_3-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetic acids, their amides and nitriles. For the corresponding acids and nitriles the alternative synthetic approaches have been developed. Limitations of synthetic approaches concerning the synthesis of the target compounds have been also discussed. Thus, it has been shown that amides of 2-[8-R_1-9-R_2-10-R_3-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c] quinazoline-6-yl)thio]acetic acids derivatives can not be prepared by amonolysis of the corresponding ester due to the low reactivity of the compounds mentioned. It has been also stated that the synthesis of nitriles via dehydration of proper amides with phosphorous-oxychloride in dichlormethane was not successful in all cases. This fact was caused by low yields and problems with isolation of the target compounds from the reaction mixture. The structures of the compounds synthesized have been confirmed by ^{1}H, ^{13}C NMR, LC–MS analysis. The compounds synthesized have been tested for the antimicrobial and antifungal activity using standard test cultures: Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and Candida albicans ATCC 885-653. It has been shown that the compounds synthesized exhibit a high antimicrobial activity against St. aureus (compounds 3.3-3.6, 4.3-4.6, 4.7, 4.8, 4.13-4.16; MIC 12.5-25 μg/ml) and C. albicans (compounds 4.13, 4.14; MIC 12.5 μg/ml). The "structure-activity" relationship has been discussed.

Quinazoline derivatives play an important role in the present list of medicines. First of all, the compounds mentioned are known as anticancer (afatinib, vandetanib, lapatinib, trimetrexate, gefitinib, raltitrexed), antifungal (albaconazol), antibacterial (nifurquinazol) drugs, etc. [3]. Some recent publications are devoted to the search of chemotherapeutics among condensed quinazolines, in particular benzimidazo[1,2-*c*]-, benzthiadiazolimidazo[1,2-*c*]-, triazolo[1,5-*c*]-, imidazo, [1,2,4]triazolo[4,3-*c*]-, triazino[2,3-*c*]quinazolines [1, 2, 4, 5, 7, 8, 10, 13-18]. Highly effective antimicrobial, antifungal, anticancer agents have been found among the compounds mentioned. Following to the strategy aimed at the directed search of biologically active compounds among S-substituted 3-R-6-thioxo-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones [6-8, 11, 12], we were interested in chemical modification of the carboxylic group, aryl group in position 3 and introduction of halogen- and alkyl- substituents in positions 8, 9, 10 of the structure of the given compounds and evaluation of their antimicrobial and antifungal activity. The data obtained were used to understand the "structure-activity" relationship.

Experimental Part

1. Chemistry

1.1. General methods

Melting points were determined in open capillary tubes and were uncorrected. The elemental analyses (C, H, N, S) were performed using an ELEMENTAR vario EL Cube analyzer (USA). Analyses were indicated by the symbols of the elements or functions within ±0.3% of the theoretical values. ^{1}H NMR spectra (500 MHz) and ^{13}C NMR spectra (100 MHz) were recorded on a Varian-Mercury 400 (Varian Inc., Palo Alto, CA, USA) spectrometer with TMS as an internal standard in DMSO-d_6 solution. LC-MS were recorded using the chromatography/mass spectrometric system consisting of an "Agilent 1100 Series" high performance liquid chromatograph (Agilent, Palo Alto, CA, USA) equipped with a diode-matrix and an "Agilent LC/MSD SL" mass-selective detector (atmospheric pressure chemical ionization – APCI). Electron impact mass spectra (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV (Varian, USA). The purity of all compounds was checked by ^{1}H-NMR and LC-MS.

Substances **1.1-1.19** and **2.1-2.19** were synthesized according to the procedures reported [7, 8]. Other starting

materials and solvents were obtained from commercially available sources and used without additional purification.

1.2. General procedure for the synthesis of [(8-R_1-9-R_2-10-R_3-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetic acids (3.1-3.14).

Method A. Add the solution of 0.94 g (10 mmol) chloracetic acid with 0.40 g (10 mmol) of sodium hydroxide in 5 ml of water to the solution of potassium of the proper 8-R_1-9-R_2-10-R_3-3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]guinazoline-6-thiolates (**2.1-2.18**) (10 mmol) in 20 ml of water, reflux for 1.5 hours to the neutral pH. Then add 50 ml of water to the resulted mixture and filter. Acidify a filtrate with hydrochloric acid to pH 3. Filter the solid obtained and dry. Recrystallize compounds from dioxane.

Method B. Add the proper 8-R_1-9-R_2-10-R_3-3-R-6-thio-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]guinazoline-2-ones (**1.1-1.18**) (5 mmol) and 0.47 g (5 mmol) of chloracetic acid to the solution of 0.23 g (10 mmol) of metallic sodium in 20 ml of ethanol, reflux for 1.5 hours to the neutral pH. Then add 50 ml of water to the resulted mixture and filter. Acidify a filtrate with hydrochloric acid to pH 3. Filter the solid obtained and dry. Recrystallize compounds from dioxane.

2-[(3-Methyl-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.1). Yield – 71.9% (method A), 69.4% (method B). M.p. – 238-240°C; ^{1}H NMR: δ = 2.36 (s, 3H, CH_3), 4.06 (s, 2H, -SC*H*$_2$), 7.68-7.59 (m, 2H, H-8, 10), 7.93 (t, 1H, *J* = 7.9, H-9), 8.41 (d, 1H, *J* = 7.9, H-11), 12.90 (s, 1H, COOH); ^{13}C NMR: δ = 18.19 (CH_3), 34.21 (SCH_2), 118.50 (11a), 126.00 (8), 126.76 (10), 128.01 (11), 136.02 (9), 144.09 (11b), 151.93 (3), 154.41 (6), 155.28 (7a), 160.98 (2), 170.04 (COOH); LC-MS, *m/z* = 303 [M+1], 304 [M+2]; Anal. Calcd for $C_{13}H_{10}N_4O_3S$: C, 51.65; H, 3.33; N, 18.53; S, 10.61; Found: C, 51.66; H, 3.34; N, 18.52; S, 10.61.

2-[(3-Phenyl-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.2). Yield – 75.8% (method A), 70.3% (method B). M.p. – 270-272°C; ^{1}H NMR: δ = 4.14 (s, 2H, -SCH_2), 7.66-7.58 (m, 3H, H-3′, 4′, 5′), 7.74-7.68 (m, 2H, H-8, 10), 7.98 (t, 1H, *J* = 7.9, H-9), 8.28 (d, 2H, *J* = 8.2, H-2′, 6′), 8.49 (d, 1H, *J* = 7.9, H-11), 12.97 (s, 1H, COOH); LC-MS, *m/z* = 307 [M+1], 309 [M+3]; Anal. Calcd for $C_{18}H_{12}N_4O_3S$: C, 59.33; H, 3.32; N, 15.38; S, 8.80; Found: C, 59.33; H, 3.33; N, 15.37; S, 8.81.

2-[(3-(4′-Methylphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.3). Yield – 84.9% (method A) and 80.6% (method B). M.p. – 234-236°C; ^{1}H NMR: δ = 2.38 (s, 3H, CH_3), 4.10 (s, 2H, -SCH_2), 7.37 (d, 2H, *J* = 8.2, H-3′, 5′), 7.71-7.61 (m, 2H, H-8, 10), 7.94 (t, 1H, *J* = 7.9, H-9), 8.20 (d, 2H, *J* = 8.2, H-2', 6'), 8.44 (d, 1H, *J* = 7.9, H-11), 12.93 (s, 1H, COOH); LC-MS, *m/z* = 321 [M–CH_2COOH]$^+$, 379 [M+1], 381 [M+3]; Anal. Calcd for $C_{19}H_{14}N_4O_3S$: C, 60.31; H, 3.73; N, 14.81; S, 8.47; Found: C, 60.32; H, 3.73; N, 14.81; S, 8.49.

2-[(3-(3′,4′-Dimethylphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.4). Yield – 85.4% (method A), 80.9% (method B). M.p. – 226-228°C; ^{1}H NMR: δ = 2.31 (d, 6H, *J* = 4.1, 3,4-$(CH_3)_2$), 4.11 (s, 2H, -SCH_2), 7.33 (d, 1H, *J* = 8.1, H-5′), 7.72-7.64 (m, 2H, H-10, 8), 7.95 (t, 1H, *J* = 7.9, H-9), 8.04 (d, 1H, *J*=8.1, H-6'), 8.07 (s, 1H, H-2′), 8.46 (d, 1H, *J* = 7.9, H-11), 12.99 (s, 1H, COOH); LC-MS, *m/z* = 335 [M–CH_2COOH]$^+$, 393 [M+1], 395 [M+3]; Anal. Calcd for $C_{20}H_{16}N_4O_3S$: C, 61.21; H, 4.11; N, 14.28; S, 8.17; Found: C, 61.23; H, 4.13; N, 14.29; S, 8.18.

2-[(3-(4′-Ethylphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.5). Yield – 75.9%. M.p. – 161-163°C; ^{1}H NMR δ: 1.31 (t, *J* = 7.5 Hz, 3H, $CH_2\underline{CH_3}$), 2.77 (q, *J* = 7.3 Hz, 2H, $\underline{CH_2}CH_3$), 4.07 (s, 2H, -SCH_2), 7.37 (d, *J* = 8.2 Hz, 2H, 3-Ph H-3′, 5′), 7.65 (t, *J* = 7.6 Hz, 1H, H-10), 7.75 (d, *J* = 8.0 Hz, 1H, H-8), 7.93 (t, *J* = 7.5 Hz, 1H, H-9), 8.28 (d, *J* = 8.0 Hz, 2H, 3-Ph H-2′, 6′), 8.53 (d, *J* = 7.9 Hz, 1H, H-11), 12.79 (s, 1H, COOH); LC-MS, m/z = 393 [M+1], 395 [M+3]; Anal. calcd. for $C_{20}H_16N_4O_3S$: C, 61.21; H, 4.11; N, 14.28; S, 8.17; Found: C, 61.24; H, 4.11; N, 14.27; S, 8.19.

2-[(3-(4′-*i*-Propylphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.6). Yield – 43.3%. M.p. – 236-238°C; ^{1}H NMR δ: 1.32 (d, *J* = 6.8 Hz, 6H, $CH(\underline{CH_3})_2$), 3.02 (dt, *J* = 13.3, 6.5 Hz, 1H, $\underline{CH}(CH_3)_2$), 4.05 (s, 2H, -SCH_2), 7.38 (d, *J* = 8.1 Hz, 2H, 3-Ph H-3′, 5′), 7.63 (t, *J* = 7.5 Hz, 1H, H-10), 7.75 (d, *J* = 8.0 Hz, 1H, H-8), 7.91 (t, *J* = 7.5 Hz, 1H, H-9), 8.29 (d, *J* = 8.1 Hz, 2H, 3-Ph H-2′, 6′), 8.54 (d, *J* = 7.9 Hz, 1H, H-11), 12.82 (s, 1H, COOH); LC-MS, m/z = 407 [M+1], 409 [M+3]; Anal. calcd. for $C_{21}H_{18}N_4O_3S$: C, 62.05; H, 4.46; N, 13.78; S, 7.89; Found: C, 62.07; H, 4.45; N, 13.79; S, 7.88.

2-2[(3-(4′-Methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.7). Yield – 77.4% (method A), 70.3% (method B). M.p. – 238-242°C; ^{1}H NMR: δ = 3.84 (s, 3H, OCH_3), 4.10 (s, 2H, -SCH_2), 7.11 (d, 2H, *J*=8.8, H-3′, 5′), 7.72-7.60 (m, 2H, H-10, 8), 7.93 (t, 1H, *J* = 7.9, H-9), 8.34 (d, 2H, *J*=8.8, H-2', 6'), 8.44 (d, 1H, *J* = 7.9, H-11), 12.89 (s, 1H, COOH); ^{13}C NMR: δ = 34.22 (SCH_2), 55.94 (OCH_3), 114.45 (3′,5′-Ph), 118.21 (11a), 124.27 (8), 126.00 (10), 126.84 (1′-Ph), 128.04 (11), 131.70 (2′,6′-Ph), 135.90 (9), 144.04 (11b), 148.79 (3), 150.66 (6), 154.63 (7a), 160.20 (2), 162.55 (4′-Ph), 170.08 (COOH); LC-MS, *m/z* = 395 [M+1], 397 [M+3]; Anal. Calcd for $C_{19}H_{14}N_4O_4S$: C, 57.86; H, 3.58; N, 14.21; S, 8.13; Found: C, 57.85; H, 3.54; N, 14.20; S, 8.12.

2-[(3-(4′-Ethoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.8). Yield – 83.6%. M.p. – 245-247°C; LC-MS, m/z = 409 [M+1], 411 [M+3]; Anal. calcd. for $C_{20}H_{16}N_4O_4S$: C, 58.81; H, 3.95; N, 13.72; S, 7.85; Found: C, 58.80; H, 3.95; N, 13.74; S, 7.87.

2-[(3-(4′-Fluorophenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.9). Yield – 76.2%. M.p. – 230-232°C; ^{1}H NMR δ: 4.09 (s, 2H, -SCH_2), 9.46-6.62 (m, 8H, H-8, 9, 10, 11, 3-Ph H-2′, 3′, 5′, 6′), 12.83 (s, 1H, COOH); LC-MS, m/z = 383 [M+1], 385 [M+3]; Anal. calcd. for $C_{18}H_{11}FN_4O_3S$: C, 56.54; H, 2.90; N, 14.65; S, 8.39; Found: C, 56.57; H, 2.90; N, 14.64; S, 8.38.

2-[(8-Methyl-3-phenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.10). Yield – 78.5%. M.p. – 255-257°C; ^{1}H NMR δ: 2.65 (s, 3H, CH_3), 4.05 (s, 2H, -SCH_2), 7.66-7.41 (m, 4H, H-10, 3-Ph H-3′, 4′, 5′), 7.76 (d, *J* = 6.9 Hz, 1H, H-9), 8.46-8.25 (m, 3H, H-11, 3-Ph H-2′, 6′), 12.72 (s, 1H, COOH); LC-MS, m/z = 379 [M+1], 381 [M+3]; Anal. calcd. for $C_{19}H_{14}N_4O_3S$: C, 60.31; H, 3.73; N, 14.81; S, 8.47; Found: C, 60.34; H, 3.73; N, 14.80; S, 8.45.

2-[(9-Fluoro-3-phenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.11). Yield –

75.9%. M.p. – 238-240ºC; ^{1}H NMR δ: 4.08 (s, 2H, -SCH_2), 7.68-7.39 (m, 5H, H-8, 10, 3-Ph H-3′, 4′, 5′), 8.32 (d, *J* = 7.0 Hz, 2H, 3-Ph H-2′, 6′), 8.60 (dd, *J* = 8.40, 6.10 Hz, 1H, H-11), 12.94 (s, 1H, COOH); LC-MS, m/z = 383 [M +1], 385 [M +3]; Anal. calcd. for $C_{18}H_{11}FN_4O_3S$: C, 56.54; H, 2.90; N, 14.65; S, 8.39; Found: C, 56.51; H, 2.90; N, 14.64; S, 8.41.

2-[(9-Fluoro-3-(4′-fluorophenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.12). Yield – 50.6%. M.p. – 247-249ºC; ^{1}H NMR δ: 4.08 (s, 2H, -SCH_2), 7.29 (t, *2*H, 3-Ph H-3′, 5′), 7.47-7.40 (m, 2H, H-8, 10), 8.42 (t, 2H, 3-Ph H-2′, 6′), 8.48 (t, 1H, H-11); LC-MS, m/z = 401 [M+1], 403 [M+3]; Anal. calcd. for $C_{18}H_{10}F_2N_4O_3S$: C, 54.00; H, 2.52; N, 13.99; S, 8.01; Found: C, 54.03; H, 2.52; N, 13.98; S, 8.03.

2-[(10-Chloro-3-phenyl)-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.13). Yield – 64.6%. M.p. – 258-260ºC; ^{1}H NMR δ: 4.07 (s, 2H, -SCH_2), 7.65-7.39 (m, 3H, 3-Ph H-3′,4′, 5′), 7.76 (d, *J* = 8.2 Hz, 1H, H-8), 7.91 (d, *J* = 8.2 Hz, 1H, H-9), 8.50-8.22 (m, 3H, H-11, 3-Ph H-2′, 6′), 13.63 (s, 1H, COOH); LC-MS, m/z = 399 [M+1], 401 [M+3], 402 [M+4]; Anal. calcd. for $C_{18}H_{11}ClN_4O_3S$: C, 54.21; H, 2.78; N, 14.05; S, 8.04; Found: C, 54.24; H, 2.78; N, 14.03; S, 8.02.

2-[(10-Bromo-3-(4′-fluorophenyl)-2-oxo-2*H*-[1,2,4] triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (3.14). Yield – 70.5%. M.p. – 257-259ºC; LC-MS, m/z = 461 $[M]^+$, 465 [M+4]; Anal. calcd. for $C_{18}H_{10}BrFN_4O_3S$: C, 46.87; H, 2.19; N, 12.15; S, 6.95; Found: C, 46.88; H, 2.19; N, 12.16; S, 6.93.

1.3. General procedure for the synthesis of N-R_4-[(8-R_1-9-R_2-10-R_3-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c] quinazoline-6-yl)thio]acetamides (4.1-4.26). Add 0.93 g (10 mmol) of chloracetamide or 10 mmol N-R_4-chloracetamides to the suspension of potassium of the proper 8-R_1-9-R_2-10-R_3-3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*] guinazoline-6-thiolates (**2.1-2.18**) (10 mmol) in 20 ml of propanol-2 and reflux for 1-1.5 hours. Cool the resulted mixture, filter the solid and dry. Crystallize the compounds obtained from dioxane–water mixture.

2-[(3-Methyl-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.1). Yield – 87.3%. M.p. – 289-291ºC; ^{1}H NMR δ: 2.45 (s, 3H, CH_3), 3.93 (s, 2H, -SCH_2), 7.08 (s, 1H, NH_2), 7.50 (s, 1H, NH_2), 7.62 (t, *J* = 8.2 Hz, 1H, H-10), 7.77 (d, *J* = 8.2 Hz, 1H, H-8), 7.91 (t, *J* = 7.3 Hz, 1H, H-9), 8.52 (d, *J* = 7.5 Hz, 1H, H-11); LC-MS, m/z = 302 [M+1], 304[M+3]; Anal. calcd. for $C_{13}H_{11}N_5O_2S$: C, 51.82; H, 3.68; N, 23.24; S, 10.64; Found: C, 51.82; H, 3.69; N, 23.24; S, 10.63.

2-[(3-Phenyl-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.2). Yield – 82.3%. M.p. – 285-288ºC; ^{1}H NMR δ: 3.98 (s, 2H, -SCH_2), 7.12 (s, 1H, NH_2), 7.61-7.43 (m, 4H, 3-Ph 3,5, NH_2), 7.66 (t, *J* = 7.6 Hz, 1H, H-10), 7.80 (d, *J* = 7.7 Hz, 1H, H-8), 7.94 (t, *J* = 7.1 Hz, 1H, H-9), 8.36 (d, *J* = 7.2 Hz, 2H, 3-Ph H-2,6), 8.56 (d, *J* = 7.6 Hz, 1H, H-11); LC-MS, m/z = 366 [M+1]; Anal. calcd. for $C_{18}H_{13}N_5O_2S$: C, 59.49; H, 3.61; N, 19.27; S, 8.82; Found: 59.48; H, 3.61; N, 19.27; S, 8.83.

2-[(3-(4-Methylphenyl)-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetamide (4.3). Yield – 69.3%. M.p. – 264-267ºC; IR (cm^{-1}): 3434, 3314, 1682, 1664, 1589, 1561, 1543, 1496, 1469, 1400, 1367, 1341, 1311, 1272, 1240, 1190, 1161, 1135, 1108, 1075, 1021, 991, 940, 885, 833, 784, 772, 707, 686, 643, 629; ^{1}H NMR δ: 2.39 (s, 3H, CH_3), 4.00 (s, 2H, -SCH_2), 7.30 (s, 1H, -C(O)NH_2), 7.39 (d, 2H, *J* = 8.2, H-3′, 5′ Ph), 7.78-7.64 (m, 3H, H-8, 10, -C(O)NH_2), 7.96 (t, 1H, *J* = 7.9, H-9), 8.22 (d, 2H, *J* = 8.2, H-2′, 6′ Ph), 8.46 (d, 1H, *J* = 7.9, H-11); LC-MS, m/z = 378 [M+1], 380 [M+3]; Anal. calcd. for $C_{19}H_{15}N_5O_2S$: C, 60.47; H, 4.01; N, 18.56, S, 8.50; Found: C, 59.47; H, 4.03; N, 18.55; S, 8.52.

2-[(3-(3,4-Dimethylphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.4). Yield – 94.05%. M.p. – 272-275ºC; ^{1}H NMR δ: 2.33 (s, 3H, 3-CH_3), 2.37 (s, 3H, 4-CH_3), 3.95 (s, 2H, -SCH_2), 7.07 (s, 1H, NH_2), 7.51 (s, 1H, NH_2), 7.28 (d, *J* = 7.9 Hz, 1H, 3-Ph H-5′), 7.63 (t, *J* = 8.2 Hz, 1H, H-10), 7.76 (d, *J* = 8.2 Hz, 1H, H-8), 7.90 (t, *J* = 7.3 Hz, 1H, H-9), 8.03 (d, *J* = 7.8 Hz, 1H, 3-Ph H-6′), 8.12 (s, 1H, 3-Ph H-2′), 8.53 (d, *J* = 7.5 Hz, 1H, H-11); LC-MS, m/z =392 [M+1], 394 [M+4]; Anal. calcd. for $C_{20}H_{17}N_5O_2S$: C, 61.37; H, 4.38; N, 17.89; S, 8.19; Found: C, 61.36; H, 4.38; N, 17.89; S, 8.20.

2-[(3-(4′-Ethylphenyl)-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetamide (4.5). Yield – 86.4%. M.p. – 233-235ºC; ^{1}H NMR δ: 1.30 (t, J = 7.4 Hz, 3H CH_3), 2.75 (dd, J = 14.8, 7.2 Hz, 2H, CH_2CH_3), 3.97 (s, 2H, -SCH_2), 7.13 (s, 1H, NH_2), 7.35 (d, J = 8.0 Hz, 2H, 3-Ph H-3, 5), 7.54 (s, 1H, NH_2), 7.63 (t, J = 7.5 Hz, 1H, H-10), 7.78 (d, J = 8.1 Hz, 1H, H-8), 7.91 (t, J = 7.3 Hz, 1H, H-9), 8.29 (d, J = 8.0 Hz, 2H, 3-Ph H-2,6), 8.53 (d, J = 7.8 Hz, 1H, H-11); LC-MS, m/z = 392 [M+1], 394 [M+3]; Anal. calcd. for $C_{20}H_{17}N_5O_2S$: C, 61.37; H, 4.38; N, 17.89; S, 8.19; Found: C, 61.39; H, 4.38; N, 17.88; S, 8.18.

2-[(3-(4′-(*tert*-Butyl)phenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.6). Yield – 79.5%. M.p. – 265-268ºC; ^{1}H NMR δ: 1.39 (s, 9H, $C(CH_3)_3$), 3.97 (s, 2H, -SCH_2), 7.13 (s, 1H, NH_2), 7.54 (d, *J* = 7.9 Hz, 3H, 3-Ph H-3,5, NH_2), 7.62 (t, *J* = 7.4 Hz, 1H, H-10), 7.77 (d, *J* = 8.0 Hz, 1H, H-8), 7.90 (t, *J* = 7.3 Hz, 1H, H-9), 8.30 (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 = 420 [M+1], 422 [M+3]; Anal. calcd. for $C_{22}H_{21}N_5O_2S$: C, 62.99; H, 5.05; N, 16.69; S, 7.64; Found: C, 62.99; H, 5.06; N, 16.69; S, 7.63.

2-[(3-(4′-Methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.7). Yield – 88.9%. M.p. – 253-255ºC; ^{1}H NMR δ: 3.90 (s, 3H, CH_3), 3.97 (s, 2H, -SCH_2), 7.07 (d, *J* = 8.2 Hz, 2H, 3-Ph H-3′, 5′), 7.13 (s, 1H NH_2), 7.55 (s, 1H, NH_2), 7.64 (t, *J* = 7.5 Hz, 1H, H-10), 7.79 (d, *J* = 7.6 Hz, 1H, H-8), 7.92 (t, *J* = 7.5 Hz, 1H, H-9), 8.42 (d, *J* = 8.2 Hz, 2H, 3-Ph H-2′,6′), 8.54 (d, *J* = 7.1 Hz, 1H, H-11); LC-MS, m/z = 394 [M+1], 396 [M+3]; Anal. calcd. for $C_{19}H_{15}N_5O_3S$: C, 58.01; H, 3.84; N, 17.80; S, 8.15; Found: C, 58.00; H, 3.84; N, 17.80; S, 8.16.

2-[(3-(4′-Ethoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetamide (4.8). Yield – 91.6%. M.p. – 288-291ºC; ^{1}H NMR δ: 1.44 (t, 3H, CH_3), 3.96 (s, 2H, -SCH_2), 4.14 (q, J = 6.3 Hz, 2H, O$\underline{CH_2}CH_3$), 6.99 (d, J = 8.0 Hz, 2H, 3-Ph H-3′, 5′), 7.14 (s, 1H, NH_2), 7.53 (s, 1H, NH_2), 7.64 (t, J = 7.6 Hz, 1H, H-10), 7.80 (d, J = 7.6 Hz, 1H, H-8), 7.93 (t, J = 7.6 Hz, 1H, H-9), 8.42 (d, J = 8.0 Hz, 2H, 3-Ph H-2′, 6′), 8.53 (d, J = 7.6 Hz, 1H, H-11); Anal. calcd. for $C_{20}H_{17}N_5O_3S$: C, 58.96; H, 4.21; N, 17.19; S, 7.87; Found: C, 58.96; H, 4.22; N, 17.19; S, 7.86.

N-(3-Fluorophenyl)-2-[(3-(4′-methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.9). Yield – 87.8%. M.p. – 274-276ºC; ^{1}H NMR δ: 3.91

(s, 3H, -OCH_3), 4.22 (s, 2H, -SCH_2), 6.78 (dd, *J* = 10.7, 8.3 Hz, 1H, 6-Ph H-4), 7.08 (d, *J* = 8.8 Hz, 2H, 3-Ph H-3′,5′), 7.44-7.22 (m, *2*H, 6-Ph H-2, 6), 7.69 – 7.54 (m, 2H, H-10, 6-Ph H-5), 7.74 (d, *J* = 8.8 Hz, 1H, H-8), 7.91 (t, *J* = 7.8, 1H, H-9), 8.44 (d, *J* = 8.2 Hz, 2H, 3-Ph H-2′,6′), 8.54 (d, *J* = 7.8 Hz, 1H, H-11), 10.53 (s, 1H, NH); LC-MS, m/z = 488 [M+1], 490 [M+3]; Anal. calcd. for $C_{25}H_{18}FN_5O_3S$: C, 61.59; H, 3.72; N, 14.37; S, 6.58; Found: C, 61.61; H, 3.72; N, 14.36; S, 6.57.

2-[(3-(4′-Fluorophenyl)-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetamide (4.10). Yield – 81.1%. M.p. – 284-287ºC; ^{1}H NMR δ: 3.95 (s, 2H, -SCH_2), 7.12 (s, 1H, NH_2), 7.29 (t, *J* = 8.5 Hz, 2H, 3-Ph H-3′,5′), 7.55 (s, 1H, NH_2), 7.71 (t, *J* = 7.4 Hz, 1H, H-10), 7.89 (d, *J* = 8.0 Hz, 1H, H-8), 7.99 (t, *J* = 7.3 Hz, 1H, H-9), 8.43 (dd, *J* = 7.5, 5.9 Hz, 2H, 3-Ph H-2′, 6′), 8.59 (d, *J* = 7.9 Hz, 1H, H-11); LC-MS, m/z = 382 [M+1], 384 [M+3]; Anal. calcd. for $C_{18}H_{12}FN_5O_2S$: C, 56.69; H, 3.17; 18.36; S, 8.41; Found: C, 56.68; H, 3.17; 18.376; S, 8.41.

2-[(8-Methyl-3-phenyl-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetamide (4.11). Yield – 86.2%. M.p. – 282-284ºC; ^{1}H NMR δ: 2.75 (s, 3H, CH_3), 3.98 (s, 2H, -SCH_2), 7.15 (s, 1H, NH_2), 7.62-7.43 (m, 5H, H-10, 3-Ph H-3′, 4′, 5′, NH_2), 7.82 (d, *J* = 5.6 Hz, 1H, H-9), 8.30 (d, *J* = 8.8 Hz, 2H, 3-Ph H-2′, 6′), 8.43 (d, *J* = 7.8 Hz, 1H, H-11); Anal. calcd. for $C_{19}H_{15}N_5O_2S$: C, 60.46; H, 4.01; N, 18.56; S, 8.50; Found: C, 60.45; H, 4.01; N, 18.56; S, 8.51.

2-[(8-Bromo-3-(4-fluorophenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.12). Yield – 79.5%. M.p. – 270-273ºC; ^{1}H NMR δ: 3.94 (s, 2H, -SCH_2), 6.60 (t, *J* = 7.6 Hz, 1H, H-10), 7.15 (s, 1H, NH_2), 7.21 (t, *J* = 7.8 Hz, 2H, 3-Ph H-3′,5′), 7.54 (s, 1H, NH_2), 7.66 (d, *J* = 7.4 Hz, 1H, H-9), 7.75 (d, *J* = 7.7 Hz, 1H, H-11), 8.33 (t, 2H, J = 5.3 Hz, 3-Ph H-2′,6′); LC-MS, m/z = 460 [M]; Anal. calcd. for $C_{18}H_{11}BrFN_5O_2S$: C, 46.97; H, 2.41; N, 15.22; S, 6.97; Found: C, 46.96; H, 2.41; N, 15.22; S, 6.98.

2-[(9-Fluoro-3-phenyl-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetamide (4.13). Yield – 91.2%. M.p. – 286-289ºC; ^{1}H NMR δ: 3.95 (s, 2H, -SCH_2), 7.13 (s, 1H, NH_2), 7.47-7.36 (t, 1H, H-10), 7.54 (m, 5H, H-8, 3-Ph H-3′, 4′, 5′, NH_2), 8.33 (d, J = 5.7 Hz, 2H, 3-Ph H-2′,6′), 8.59 (dd, J = 6.2, 4.6 Hz, 1H, H-11); LC-MS, m/z = 382 [M+1], 384 [M+3]; Anal. calcd. for $C_{18}H_{12}FN_5O_2S$: C, 56.69; H, 3.17; N, 18.36; S, 8.41; Found: C, 56.69; H, 3.16; N, 18.36; S, 8.42.

2-[(9-Fluoro-3-(4′-methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.14). Yield – 89.1%. M.p. – 251-254ºC; ^{1}H NMR δ: 3.89 (s, 3H, -OCH_3), 3.95 (s, 2H, -SCH_2), 7.05 (d, *J* = 8.6 Hz, 2H, 3-Ph H-3′, 5′), 7.14 (s, 1H, NH_2), 7.43 (dd, *J* = 8.6, 6.5 Hz, 1H, H-10), 7.51 (d, *J* = 8.7 Hz, 1H, H-8), 7.56 (s, 1H, NH_2), 8.40 (d, *J* = 8.7 Hz, 2H, 3-Ph H-2,6), 8.59 (dd, *J* = 8.0, 5.4 Hz, 1H, H-11); LC-MS, m/z = 412 [M+1], 414 [M+3]; Anal. calcd. for $C_{19}H_{14}FN_5O_3S$: C, 55.47; H, 3.43; N, 17.02; S, 7.79; Found: C, 55.43; H, 3.46; N, 17.01; S, 7.80.

N-(4-Bromophenyl)-2-[(9-fluoro-3-(4-methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio] acetamide (4.15). Yield – 92.6%. M.p. – 304-306ºC; ^{1}H NMR δ: 3.90 (s, 3H, -OCH_3), 4.20 (s, 2H, -SCH_2), 7.08 (d, *J* = 8.7 Hz, 2H, 3-Ph H-3′, 5′), 7.51-7.32 (m, 4H, H-8, 10, 6-Ph H-2′, 6′), 7.62 (d, *J* = 8.5 Hz, 2H, 6-Ph H-3′, 5′), 8.41 (d, *J* = 8.7 Hz, 2H, 3-Ph H-2′, 6′), 8.57 (dd, *J* = 8.4, 6.3 Hz, 1H, H-11), 10.46 (s, 1H, NH); Anal. calcd. for $C_{25}H_{17}BrFN_5O_3S$: C, 53.01; H, 3.03; Br, 14.11; F, 3.35; N, 12.36; S, 5.66; Found: C, 53.04; H, 3.03; Br, 14.11; F, 3.35; N, 12.34; S, 5.65.

2-[(9-Fluoro-3-(4-fluorophenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.16). Yield – 71.8%. M.p. – 274-276ºC; ^{1}H NMR δ: 3.96 (s, 2H, -SCH_2); 7.17 (s, 1H, NH_2), 7.28 (t, *J* = 8.4 Hz, 2H, 3-Ph H-3′,5′), 7.53 (t, *J* = 7.5 Hz, 1H, H-10), 7.55 (s, 1H, NH_2), 7.56 (d, *J* = 9.2 Hz, 1H, H-8), 8.39 (t, *J* = 5.7 Hz, 2H, 3-Ph H-2′,6′), 8.65 (dd, *J* = 8.3, 6.0 Hz, 1H, H-11); LC-MS, m/z = 400 [M+1], 402 [M+3]; Anal. calcd. for $C_{18}H_{11}F_2N_5O_2S$: C, 54.13; H, 2.78; N, 17.54; S, 8.03; Found: C, 54.12; H, 2.78; N, 17.55; S, 8.03.

2-[(9-Bromo-3-(4-fluorophenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.17). Yield – 84.62%. M.p. – 282-285ºC; ^{1}H NMR δ: 3.95 (s, 2H, -SCH_2); 7.12 (s, 1H, NH_2), 7.30 (t, *J* = 8.4 Hz, 2H, 3-Ph H-3,5), 7.75 (d, *J* = 8.1 Hz, 1H, H-10), 7.55 (s, 1H, NH_2), 8.00 (s, 1H, H-8), 8.55-8.32 (m, 3H, H-11, 3-Ph H-2,6); LC-MS, m/z = 461 [M+1]; Anal. calcd. for $C_{18}H_{11}BrFN_5O_2S$: C, 46.97; H, 2.41; N, 15.22; S, 6.97; Found: C, 46.97; H, 2.42; N, 15.22; S, 6.96.

N-(4-(Trifluoromethyl)benzyl)-2-[(9-bromo-3-(4-fluorophenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.18). Yield – 81.9%. M.p. – 273-276ºC; ^{1}H NMR δ: 4.04 (s, 2H, -SCH_2), 4.44 (d, *J* = 4.6 Hz, 2H, -CH_2), 7.33 (t, *J* = 7.7 Hz, 2H, 3-Ph H-3′, 5′), 7.57-7.41 (m, 4H, 6-Ph H-2′, 3′, 5′, 6′), 7.79 (d, *J* = 8.4 Hz, 1H, H-10), 7.90 (s, 1H, H-8), 8.51-8.33 (m, 3H, H-11, 3-Ph H-2′, 6′), 8.80 (t, *J* = 5.9 Hz, 1H, NHCO); Anal. calcd. for $C_{26}H_{16}BrF_4N_5O_2S$: C, 50.50; H, 2.61; Br, 12.92; F, 12.29; N, 11.32; S, 5.19; Found: C, 50.53; H, 2.61; Br, 12.92; F, 12.29; N, 11.31; S, 5.17.

2-[(10-Chloro-3-phenyl-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetamide (4.19). Yield – 65.9%. M.p. – 273-276ºC; ^{1}H NMR δ: 3.97 (s, 2H, -SCH_2), 7.13 (s, 1H, NH_2), 7.66-7.39 (m, 3H, 3-Ph H-3′,5′, NH_2), 7.81 (d, *J* = 8.7 Hz, 1H, H-8), 7.89 (d, *J* = 8.7 Hz, 1H, H-9), 8.36 (d, *J* = 7.1 Hz, 2H, 3-Ph H-2′, 6′), 8.47 (s, 1H, H-11); LC-MS, m/z = 398 [M+1], 400 [M+3], 401[M+4]; Anal. calcd. for $C_{18}H_{12}ClN_5O_2S$: C, 54.34; H, 3.04; N, 17.60; S, 8.06; Found: C, 54.36; H, 3.04; N, 17.59; S, 8.05.

N-(4-Methoxybenzyl)-2-[(10-chloro-2-oxo-3-phenyl-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.20). Yield – 81.4%. M.p. – 242-244ºC; ^{1}H NMR δ: 3.74 (s, 3H, OCH_3), 4.01 (s, 2H, -SCH_2), 4.27 (d, *J* = 5.5 Hz, 2H, -CH_2), 6.74 (d, *J* = 8.2 Hz, 2H, 6-Ph H-3′, 5′), 7.17 (d, *J* = 8.1 Hz, 2H, 6-Ph H-2′, 6′), 7.66-7.46 (m, 4H, H-8, 3-Ph H-3′,4′, 5′), 7.86 (d, *J* = 8.6 Hz, 1H, H-9), 8.35 (d, *J* = 7.3 Hz, 2H, 3-Ph H-2′, 6′), 8.45 (s, 1H, H-11), 8.60 (t, *J* = 5.5 Hz, 1H, NH); LC-MS, m/z = 519 [M+1], 522 [M+4]; Anal. calcd. for $C_{26}H_{20}ClN_5O_3S$: C, 60.29; H, 3.89; Cl, 6.84; N, 13.52; S, 6.19; Found: C, 60.32; H, 3.89; Cl, 6.84; N, 13.50; S, 6.22.

N-(4-(Trifluoromethyl)benzyl)-2-[(10-chloro-3-(4-methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*] quinazolin-6-yl)thio]acetamide (4.21). Yield – 93.5%. M.p. – 279-282ºC; ^{1}H NMR δ: 3.91 (s, 3H, -OCH_3), 4.05 (s, 2H, -SCH_2), 4.43 (d, *J* = 3.8 Hz, 2H, -CH_2), 7.08 (d, *J* = 7.6 Hz, 2H, 3-Ph H-3′, 5′), 7.54-7.40 (m, 5H, 6-Ph H-2′,3′,4′, 5′, 6′), 7.64 (d, *J* = 7.8 Hz, 1H, H-8), 7.84 (d, *J* = 7.9 Hz, 1H, H-9), 8.51-8.33 (m, 3H, H-11, 3-Ph H-2′, 6′), 8.82 (t, *J* = 3.8 Hz, 1H, NH); Anal. calcd. for $C_{27}H_{19}ClF_3N_5O_3S$: C, 55.34; H, 3.27; Cl, 6.05; F, 9.73; N, 11.95; S, 5.47; Found: C, 55.36; H, 3.27; Cl, 6.05; F, 9.73; N, 11.94; S, 5.46.

2-[(10-Bromo-3-phenyl-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetamide (4.23). Yield – 64.0%. M.p. – 250-253ºC; ^{1}H NMR δ: 3.97 (s, 2H, -SCH_2), 7.13 (s, 1H, NH_2), 7.65-7.46 (m, 4H, 3-Ph H-3′, 4′, 5′, NH_2), 7.74 (d, *J* = 7.9 Hz, 1H, H-8), 8.02 (d, *J* = 7.8 Hz, 1H, H-9), 8.36 (d, *J* = 6.9 Hz, 2H, 3Ph H-2′,6′), 8.63 (s, 1H, H-11); LC-MS, m/z = 444 [M+2], 446 [M+4]; Anal. calcd. for $C_{18}H_{12}BrN_5O_2S$: C, 48.88; H, 2.73; N, 15.83; S, 7.25; Found: C, 48.87; H, 2.74; N, 15.83; S, 7.25.

2-[(10-Bromo-3-(4-methylphenyl)-2-oxo-2*H*-[1,2,4] triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.23). Yield – 79.5%. M.p. – 275-278ºC; ^{1}H NMR δ: 2.45 (s, 3H, -CH_3), 3.96 (s, 2H, -SCH_2), 6.32 (d, *J* = 7.9 Hz, 2H, 3-Ph H-3′, 5′), 7.13 (s, 1H, NH_2), 7.57 (s, 1H, NH_2), 7.81 (d, *J* = 8.6 Hz, 1H, H-8), 8.07 (dd, *J* = 8.6, 1.6 Hz, 1H, H-9), 8.23 (d, *J* = 8.0 Hz, 2H, 3-Ph H-2′, 6′), 8.64 (s, 1H, H-11); LC-MS, m/z = 457 [M+1]; Anal. calcd. for $C_{19}H_{14}BrN_5O_2S$: C, 50.01; H, 3.09; N, 15.35; S, 7.03; Found: C, 50.02; H, 3.09; N, 15.36; S, 7.03.

N-(2-Fluorophenyl)-2-[(10-bromo-3-(4′-methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl) thio]acetamide (4.24). Yield – 87.7%. M.p. – 284-286ºC; ^{1}H NMR δ: 3.91 (s, 3H, -OCH_3), 4.26 (s, 2H, -SCH_2), 7.27-7.08 (m, 5H, 3-Ph H-3′, 5′, 6-Ph H-3′, 4′, 5′), 7.70 (d, *J* = 8.8 Hz, 1H, 6-Ph H-6′), 8.10-7.88 (m, 2H, H-8, 9), 8.43 (d, *J* = 8.7 Hz, 2H, 3-Ph H-2′, 6′), 8.61 (s, 1H, H-11), 10.00 (s, 1H, NH); LC-MS, m/z = 567 [M+1]; Anal. calcd. for $C_{25}H_{17}BrFN_5O_3S$: C, 53.01; H, 3.03; Br, 14.11; F, 3.35; N, 12.36; S, 5.66; Found: C, 53.04; H, 3.03; Br, 14.11; F, 3.35; N, 12.34; S, 5.65.

N-(3-Fluorophenyl-)2-[(10-bromo-3-(4′-methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl) thio]acetamide (4.25). Yield – 81.7%. M.p. – 288-290ºC; ^{1}H NMR δ: 3.91 (s, 3H, -OCH_3), 4.21 (s, 2H, -SCH_2), 6.80 (dd, *J* = 9.4, 7.8 Hz, 1H, 6-Ph H-4′), 7.09 (d, *J* = 7.6 Hz, 2H, 3-Ph H-2′, 5′), 7.42-7.23 (m, 2H, 6-Ph H-5′, 6′), 7.59 (dd, *J* = 11.3, 0.7 Hz, 1H, 6-Ph H-2′), 7.67 (d, *J* = 7.8 Hz, 1H, H-8), 8.01 (d, *J* = 7.8 Hz, 1H, H-9), 8.43 (d, *J* = 8.0 Hz, 2H, 3-Ph H-2′, 6′), 8.53 (s, 1H, H-11), 10.53 (s, 1H, NH); LC-MS, m/z = 568 [M+2]; Anal. calcd. for $C_{25}H_{17}BrFN_5O_3S$: C, 53.01; H, 3.03; Br, 14.11; F, 3.35; N, 12.36; S, 5.66; Found: C, 53.04; H, 3.03; Br, 14.11; F, 3.35; N, 12.34; S, 5.65.

2-[(10-Bromo-3-(4′-fluorophenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamide (4.26). Yield – 66.7%. M.p. – 289-291ºC; LC-MS, m/z = 463 [M+3]; Anal. calcd. for $C_{18}H_{11}BrFN_5O_2S$: C, 46.97; H, 2.41; N, 15.22; S, 6.97; Found: C, 46.96; H, 2.41; N, 15.22; S, 6.98.

1.4. General procedure for the synthesis of 2-[(8-R_1-9-R_2-10-R_3-3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetonitriles (5.1-5.18).

Method A. Add 0.75 g (10 mmol) of chloroacetonitrile to the suspension of the proper potassium 8-R_1-9-R_2-10-R_3-3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]guinazoline-6-thiolates (2.1-2.18) (10 mmol) in 20 ml of propanol-2 and reflux for 1-1.5 hours. Cool the resulted mixture, filter the solid and dry. Crystallize the compounds from DMF-water.

Method B. Stir the mixture of 10 mmol of the proper 2-[(8-R_1-9-R_2-10-R_3-3-R-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazoline-6-yl)thio]acetamides (4.2, 4.5, 4.8, 4.12), 0.35 g (0.6 mmol) NaCl, 25 ml anhydrous dichloroethane, 1.0 g (6.5 mmol) phosphorous oxychloride and 1-2 drops of pyridine when heating (84ºC) for 50 min. Then increase the temperature to 88°C and continue to stir for 4 hours. Evaporate the solvent under vacuum, recrystallize the solid obtained from DMA-water.

2-[(3-Methyl-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetonitrile (5.1). Yield – 99.3% (Method A). M.p. – 279-281ºC; ^{1}H NMR δ: 2.44 (s, 3H, CH_3), 4.28 (s, 2H, -SCH_2), 7.68 (t, *J* = 7.4 Hz, 1H, H-10), 7.86 (d, *J* = 8.2 Hz, 1H, H-8), 7.97 (t, *J* = 8.2 Hz, 1H, H-9), 8.56 (d, *J* = 7.8 Hz, 1H, H-11); LC-MS, m/z = 284 [M+1], 286 [M+3]; Anal. calcd. for $C_{13}H_9N_5OS$: C, 55.11; H, 3.20; N, 24.72; S, 11.32; Found: C, 55.10; H, 3.20; N, 24.72; S, 11.33.

2-[(3-Phenyl-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.2). Yield – 99.6% (Method A); 68.7% (Method B). M.p. – 257-259ºC; ^{1}H NMR δ: 4.33 (s, 2H, -SCH_2), 7.63-7.48 (m, 3H, 3-Ph H-3′, 4′, 5′), 7.70 (t, *J* = 7.5 Hz, 1H, H-10), 7.87 (d, *J* = 7.8 Hz, 1H, H-8), 7.98 (t, *J* = 7.4 Hz, 1H, H-9), 8.32 (d, *J* = 7.8 Hz, 2H, 3-Ph H-2′, 6′), 8.58 (d, *J* = 7.8 Hz, 1H, H-11); LC-MS, m/z = 346 [M+1], 348 [M+3]; Anal. calcd. for $C_{18}H_{11}N_5OS$: C, 62.60; H, 3.21; N, 20.28; S, 9.28; Found: C, 62.62; H, 3.20; N, 20.28; S, 9.27.

2-[(3-(3′,4′-Dimethylphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.3). Yield – 99.7% (Method A). M.p. – 258-261ºC; ^{1}H NMR δ: 2.35 (s, 3H, 3-Ph 3-CH_3), 2.38 (s, 3H, 3-Ph 4-CH_3), 4.31 (s, 2H, -SCH_2), 7.26 (d, *J* = 7.9 Hz, 1H, 3-Ph H-5′), 7.70 (t, *J* = 7.4 Hz, 1H, H-10), 7.87 (d, *J* = 8.0 Hz, 1H, H-8), 7.97 (t, *J* = 7.4 Hz, 1H, H-9), 8.05 (d, *J* = 7.8 Hz, 1H, 3-Ph H-6′), 8.10 (s, 1H, 3-Ph H-2′), 8.57 (d, *J* = 7.8 Hz, 1H, H-11); LC-MS, m/z = 374 [M+1], 376 [M+3]; Anal. calcd. for $C_{20}H_{15}N_5OS$: C, 64.33; H, 4.05; N, 18.75; S, 8.59; Found: C, 64.34; H, 4.05; N, 18.74; S, 8.59.

2-[(3-(4′-*i*-Propylphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.4). Yield – 99.9% (Method A). M.p. – 176-178ºC; ^{1}H NMR δ: 1.32 (d, *J* = 5.5 Hz, 6H, -$CH(\underline{CH_3})_2$), 2.59-2.53 (m, 1H, -$\underline{CH}(CH_3)_2$), 4.32 (s, 2H, -SCH_2), 7.37 (d, *J* = 7.8 Hz, 2H, 3-Ph H-3′,5′), 7.69 (t, *J* = 7.3 Hz, 1H, H-10), 7.87 (d, *J* = 7.9 Hz, 1H, H-8), 7.97 (t, *J* = 7.2 Hz, 1H, H-9), 8.25 (d, *J* = 7.7 Hz, 2H, 3-Ph H-2′, 6′), 8.57 (d, *J* = 7.7 Hz, 1H, H-11); LC-MS, m/z =388 [M+1], 390 [M+3]; Anal. calcd. for $C_{21}H_{17}N_5OS$: C, 65.10; H, 4.42; N, 18.08; S, 8.28; Found: C, 65.10; H, 4.41; N, 18.09; S, 8.28.

2-[(3-(4′-(*tert*-Butyl)phenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.5). Yield – 96.0% (Method A); 73.7% (Method B). M.p. – 256-258ºC; ^{1}H NMR δ: 1.39 (s, 9H, -$C(\underline{CH_3})_3$); 4.32 (s, 2H, -SCH_2), 7.53 (d, *J* = 8.0 Hz, 2H, 3-Ph H-3′, 5′), 7.69 (t, *J* = 7.4 Hz, 1H, H-10), 7.86 (d, *J* = 7.9 Hz, 1H, H-8), 7.97 (t, *J* = 7.4 Hz, 1H, H-9), 8.26 (d, *J* = 8.1 Hz, 2H, 3-Ph H-2′, 6′), 8.57 (d, *J* = 7.9 Hz, 1H, H-11); LC-MS, m/z = 402 [M+1], 404 [M+3]; Anal. calcd. for $C_{22}H_{19}N_5OS$: C, 65.81; H, 4.77; N, 17.44; O, 3.99; S, 7.99; Found: C, 65.80; H, 4.77; N, 17.44; O, 3.99; S, 8.00.

2-[(3-(4′-Methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.6). Yield – 82.0% (Method A). M.p. – 272-274ºC; ^{1}H NMR δ: 3.90 (s, 3H, -OCH_3), 4.32 (s, 2H, -SCH_2), 7.05 (d, *J* = 7.3 Hz, 2H, 3-Ph H-3′, 5′), 7.70 (t, *J* = 7.6 Hz, 1H, H-10), 7.88 (d, *J* = 7.6 Hz, 1H, H-8), 7.98 (d, *J* = 7.6 Hz, 1H, H-9), 8.39 (d, *J* = 7.6 Hz, 2H, 3-Ph H-2′, 6′), 8.58 (d, *J* = 7.4 Hz, 1H, H-11); LC-MS, m/z = 376 [M+1], 378 [M+3]; Anal. calcd. for $C_{19}H_{13}N_5O_2S$: C, 60.79; H, 3.49; N, 18.66; O, 8.52; S, 8.54; Found: C, 60.80; H, 3.49; N, 18.66; O, 8.52; S, 8.53.

2-[(3-(4′-Ethoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.7). Yield – 99.9% (Method A). M.p. – 268-270ºC; ^{1}H NMR δ: 1.45 (t, *J* = 5.6 Hz, 3H, -OCH$_2$<u>CH$_3$</u>), 4.15 (dd, *J* = 11.1, 5.4 Hz, 2H, -O<u>CH$_2$</u>CH$_3$), 4.32 (s, 2H, -SCH$_2$), 7.02 (d, *J* = 7.6 Hz, 2H, 3-Ph H-3′,5′), 7.71 (t, *J* = 7.3 Hz, 1H, H-10), 7.88 (d, *J* = 7.3 Hz, 1H, H-8), 7.98 (t, *J* = 7.3 Hz, 1H, H-9), 8.38 (d, *J* = 7.7 Hz, 2H, 3-Ph H-2′, 6′), 8.58 (d, *J* = 7.3 Hz, 1H, H-11); LC-MS, m/z = 390 [M+1], 392 [M+3]; Anal. calcd. for $C_{20}H_{15}N_5O_2S$: C, 61.68; H, 3.88; N, 17.98; S, 8.23; Found: C, 61.67; H, 3.88; N, 17.98; S, 8.24.

2-[(3-(4′-Fluorophenyl)-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.8). Yield – 99.9% (Method A); 81.3% (Method B). M.p. – 266-268ºC; ^{1}H NMR δ: 4.33 (s, 2H, -SCH$_2$), 7.29 (t, *J* = 8.5 Hz, 2H, 3-Ph H-3′,5′), 7.71 (t, *J* = 7.4 Hz, 1H, H-10), 7.89 (d, *J* = 8.0 Hz, 1H, H-8), 7.99 (t, *J* = 7.3 Hz, 1H, H-9), 8.43 (dd, *J* = 7.5, 5.9 Hz, 2H, 3-Ph H-2′, 6′), 8.59 (d, *J* = 7.9 Hz, 1H, H-11); LC-MS, m/z = 364 [M+1], 366 [M+3]; Anal. calcd. for $C_{18}H_{10}FN_5OS$: C, 59.50; H, 2.77; N, 19.27; S, 8.82; Found: C, 59.51; H, 2.77; N, 19.27; S, 8.81.

2-[(8-Methyl-3-phenyl-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.9). Yield – 98.67% (Method A). M.p. – 255-257ºC; ^{1}H NMR δ: 2.76 (s, 3H, -CH$_3$), 4.32 (s, 2H, -SCH$_2$), 7.63-7.46 (m, 4H, H-10, 3-Ph H-3′, 4′, 5′), 7.81 (d, *J* = 5.6 Hz, 1H, H-9), 8.31 (d, *J* = 8.8 Hz, 2H, 3-Ph H-2′, 6′), 8.41 (d, *J* = 7.8 Hz, 1H, H-11); LC-MS, m/z = 360 [M+1], 362 [M+3]; Anal. calcd. for $C_{19}H_{13}N_5OS$: C, 63.49; H, 3.65; N, 19.49; S, 8.92; Found: C, 63.48; H, 3.65; N, 19.49; S, 8.93.

2-[(9-Fluoro-3-phenyl-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.10). Yield – 88.0% (Method A); 71.9% (Method B). M.p. – 250-253ºC; ^{1}H NMR δ: 4.33 (s, 2H, -SCH$_2$), 7.63-7.45 (m, *J* = 14.9, 7.7 Hz, 5H, H-8, 10, 3-Ph H-3′,4′,5′), 8.29 (d, *J* = 7.2 Hz, 2H, 3-Ph H-2′,6′), 8.64 (dd, *J* = 8.7, 5.9 Hz, 1H, H-11); LC-MS, m/z = 364 [M+1], 366 [M+3]; Anal. calcd. for $C_{18}H_{10}FN_5OS$: C, 59.50; H, 2.77; N, 19.27; S, 8.82; Found: C, 59.51; H, 2.77; N, 19.26; S, 8.82.

2-[(9-Fluoro-3-(4′-methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.11). Yield – 74.67% (Method A). M.p. – 273-275ºC; ^{1}H NMR δ: 3.90 (s, 3H, -CH$_3$), 4.32 (s, 2H, -SCH$_2$), 7.04 (d, *J* = 8.2 Hz, 2H, 3-Ph H-3′,5′), 7.50 (dd, *J* = 8.6, 6.6 Hz, 1H, H-10), 7.57 (d, *J* = 7.6 Hz, 1H, H -8), 8.36 (d, *J* = 8.3 Hz, 2H, 3-Ph H-2′,6′), 8.63 (dd, *J* = 8.7, 5.9 Hz, 1H, H-11); LC-MS, m/z = 394 [M+1], 396 [M+3]; Anal. calcd. for $C_{19}H_{12}FN_5O_2S$: C, 58.01; H, 3.07; N, 17.80; S, 8.15; Found: C, 58.02; H, 3.07; N, 17.80; S, 8.14.

2-[(9-Fluoro-3-(4′-fluorophenyl)-2-oxo-2*H*-[1,2,4] triazino[2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.12). Yield – 48.0% (Method A). M.p. – 258-260ºC; ^{1}H NMR δ: 4.33 (s, 2H, -SCH$_2$), 7.29 (t, *J* = 8.4 Hz, 2H, 3-Ph H-3′,5′), 7.51 (t, *J* = 7.5 Hz, 1H, H-10), 7.58 (d, *J* = 9.2 Hz, 1H, H-8), 8.40 (t, *J* = 5.7 Hz, 2H, 3-Ph H-2′,6′), 8.63 (dd, *J* = 8.3, 6.0 Hz, 1H, H-11); LC-MS, m/z = 382 [M+1], 384 [M+3]; Anal. calcd. for $C_{18}H_9F_2N_5OS$: C, 56.69; H, 2.38; N, 18.36; S, 8.41; Found: C, 56.68; H, 2.38; N, 18.37; S, 8.41.

2-[(10-Chloro-3-phenyl-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.13). Yield – 69.33% (Method A). M.p. – 262-264ºC; ^{1}H NMR δ: 4.33 (s, 2H, -SCH$_2$), 7.65-7.37 (m, 3H, 3-Ph H-3′, 4′, 5′), 7.88 (d, *J* = 8.6 Hz, 1H, H-8), 7.95 (d, *J* = 8.6 Hz, 1H, H-9), 8.31 (d, *J* = 7.3 Hz, 2H, 3-Ph H-2′, 6′), 8.50 (s, 1H, H-11); LC-MS, m/z = 380 [M +1], 382 [M +3], 383 [M +4]; Anal. calcd. for $C_{18}H_{10}ClN_5OS$: C, 56.92; H, 2.65; N, 18.44; S, 8.44; Found: C, 56.92; H, 2.65; N, 18.43; S, 8.45.

2-[(10-Chloro-3-(4′-methylphenyl)-2-oxo-2*H*-[1,2,4] triazino[2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.14). Yield – 64.0% (Method A). M.p. – 250-253ºC; ^{1}H NMR δ: 2.46 (s, 3H, -CH$_3$), 4.32 (s, 2H, S-CH$_2$-), 7.33 (d, *J* = 7.8 Hz, 2H, 3-Ph H-3′,5′), 7.87 (d, *J* = 8.6 Hz, 1H, H-8), 7.93 (d, *J* = 8.6 Hz, 1H, H-9), 8.24 (d, *J* = 7.8 Hz, 2H, 3-Ph H-2′, 6′), 8.49 (s, 1H, H-11); Anal. calcd. for $C_{19}H_{12}ClN_5OS$: C, 57.94; H, 3.07; N, 17.78; S, 8.14; Found: C, 57.93; H, 3.07; N, 17.79; S, 8.14.

2-[(10-Chloro-3-(4′-fluorophenyl)-2-oxo-2*H*-[1,2,4] triazino[2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.15). Yield – 69.33% (Method A). M.p. – 270-272ºC; ^{1}H NMR δ: 4.33 (s, 2H, -S-CH$_2$-), 7.29 (t, *J* = 8.6 Hz, 2H, 3-Ph H-3′, 5′), 7.88 (d, *J* = 8.7 Hz, 1H, H-8), 7.95 (d, *J* = 8.5 Hz, 1H, H-9), 8.42 (dd, *J* = 8.0, 5.8 Hz, 2H, 3-Ph H-2′, 6′), 8.49 (s, 1H, H-11); LC-MS, m/z = 398 [M+1], 400 [M+3], 401 [M+4]; Anal. calcd. for $C_{18}H_9ClFN_5OS$: C, 54.35; H, 2.28; N, 17.60; S, 8.06; Found: C, 54.36; H, 2.27; N, 17.60; S, 8.06.

2-[(10-Bromo-3-phenyl-2-oxo-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.16). Yield – 96.7% (Method A). M.p. – 260-252ºC; ^{1}H NMR δ: 4.33 (s, 2H, -SCH$_2$), 7.66-7.41 (m, 3H, 3-Ph H-3′, 4′, 5′), 7.81 (d, *J* = 8.6 Hz, 1H, H-8), 8.08 (d, *J* = 8.6 Hz, 1H, H-9), 8.31 (d, *J* = 7.3 Hz, 2H, 3-Ph H-2′, 6′), 8.65 (s, 1H, H-11); LC-MS, m/z = 425 [M +1]; Anal. calcd. for $C_{18}H_{10}BrN_5OS$: C, 50.96; H, 2.38; N, 16.51; S, 7.56; Found: C, 50.97; H, 2.38; N, 16.50; S, 7.56.

2-[(10-Bromo-3-(4′-methylphenyl)-2-oxo-2*H*-[1,2,4] triazino[2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.17). Yield – 77.2% (Method A). M.p. – 268-270ºC; ^{1}H NMR δ: 2.46 (s, 3H, -CH$_3$), 4.32 (s, 2H, -SCH$_2$), 6.32 (d, *J* = 7.9 Hz, 2H, 3-Ph H-3′, 5′), 7.80 (d, *J* = 8.6 Hz, 1H, H-8), 8.06 (dd, *J* = 8.6, 1.6 Hz, 1H, H-9), 8.24 (d, *J* = 8.0 Hz, 2H, 3-Ph H-2′, 6′), 8.63 (s, 1H, H-11); LC-MS, m/z = 439 [M+1]; Anal. calcd. for $C_{19}H_{12}BrN_5OS$: C, 52.07; H, 2.76; N, 15.98; S, 7.32; Found: C, 52.06; H, 2.76; N, 15.98; S, 7.33.

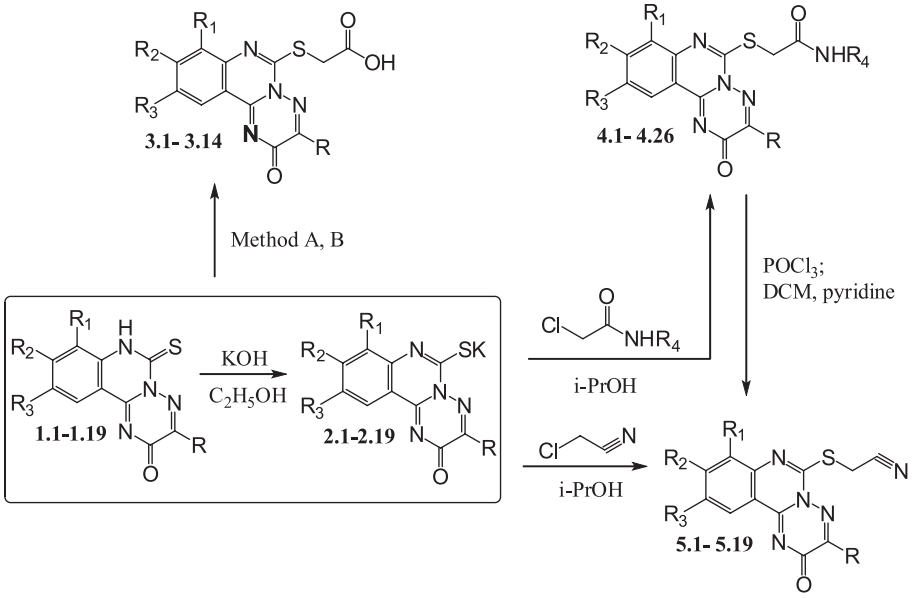
2-[(10-Bromo-3-(4′-methoxyphenyl)-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.18). Yield – 88.0% (Method A). M.p. – 266-268ºC; ^{1}H NMR δ: 3.88(s, 3H, -CH$_3$), 4.30 (s, 2H, -SCH$_2$), 7.03(d, 2H, 3-Ph H-3′, 5′), 7.81(d, 1H, H-8), 8.04 (d, 1H, H-9), 8.37 (d, 2H, 3-Ph H-2′, 6′), 8.62 (s, 1H, H-11); LC-MS, m/z = 455 [M+1], 456 [M+2], 458 [M+4]; Anal. calcd. for $C_{19}H_{12}BrN_5O_2S$: C, 50.23; H, 2.66; N, 15.42; S, 7.06; Found: C, 50.22; H, 2.66; N, 15.43; S, 7.06.

2-[(10-Bromo-3-(4′-fluorophenyl)-2-oxo-2*H*-[1,2,4] triazino[2,3-*c*]quinazolin-6-yl)thio]acetonitrile (5.19). Yield – 64.0%. M.p. – 259-261ºC; ^{1}H NMR δ: 4.33 (s, 2H, -SCH$_2$), 7.29 (t, *J* = 8.4 Hz, 3H, 3-Ph H-3′, 5′), 7.81 (d, *J* = 8.6 Hz, 1H, H-8), 8.07 (d, *J* = 8.3 Hz, 1H, H-9), 8.42 (dd, *J* = 7.3, 5.8 Hz, 2H, 3-Ph H-2′, 6′), 8.64 (s, 1H, H-11); LC-MS, m/z = 446 [M+4]; Anal. calcd. for $C_{18}H_9BrFN_5OS$: C, 48.88; H, 2.05; N, 15.84; S, 7.25; Found: C, 48.89; H, 2.05; N, 15.84; S, 7.24.

2. Pharmacology

Antimicrobial and antifungal test

Sensitivity of microorganisms to the compounds synthesized was assessed according to the methods described [9]. The assay was conducted on the Mueller-Hinton medium by two-fold serial dilution of compounds in 1 ml. After that 0.1 ml of microbial seeding (106 cells/ml) was added. The minimal inhibitory concentration of com-



Method A: $ClCH_2COOH$, NaOH, H_2O; Method B: $ClCH_2COOH$, Na, C_2H_5OH; R=C_2H_5OH; R=CH_3, Ph, 4-CH_3Ph, 3,4-$(CH_3)_2$Ph, 4-C_2H_5Ph, 4-$(CH_3)_2$CHPh, 4-$(CH_3)_3$CPh, 4-CH_3OPh, 4-C_2H_5OPh, 4-FPh; R_1=H, CH_3, Br; R_2=H, F, Br; R_3=H, Cl, Br; R_4=H, 2-FPh, 3-FPh, 4-BrPh, 4-CH_3OPh, 4-CF_3Ph

Scheme. Synthesis of [(8-R_1-9-R_2-10-R_3-3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]-quinazoline-6-yl)thio]acetic acids and the functional derivatives.

pounds was determined by the absence of visual growth in the test tube with the minimal concentration of the substance, the minimal bactericidal/fungicidal concentration was determined by the absence of growth on agar after inoculation of the microorganism from transparent test-tubes. Dimethylsulfoxide was used as a solvent, the initial solution concentration was 1 mg/ml. For preliminary screening the standard test cultures such as *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 885-653 were used. All test strains were received from the bacteriological laboratory at the Zaporizhzhya Regional Laboratory Centre of the State Sanitary and Epidemiological Service of Ukraine. Nitrofual, trimetoprim and ketoconazol were used as reference compounds with the proved antibacterial/antifungal activity. Additional quality control of the culture medium and solvents was conducted by the methods commonly used.

Results and Discussion

1. Chemistry

As initial compounds, 3-R-6-thio-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]-quinazolin-2-ones (**1**) and their potassium salts were used; the salts were obtained according to the known protocols of 6-R-3-(3-R_1-4-R_2-5-R_3-2-amnophenyl)-1,2,4-triazin-5(2H)-ones with carbon disulfide, potassium hydroxide in the ethyl alcohol medium and potassium ethylxanthate in propanol-2 [7, 8].

The synthesis of [(8-R_1-9-R_2-10-R_3-3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetic acids (**3**) was conducted via alkylation of potassium thiolates with chloroacetic acids in propanol-2 or propanol-2 – water in the presence of the equivalent amount of sodium hydroxide (Scheme). After acidification of the reaction mixture (pH 3-4), the solids of the corresponding acids were formed with high yields. Amides **4** were synthesized by alkylation of potassium thiolates **2** with chloracetamide or N-R_4-chloracetamides in propanol-2. Unfortunately, we failed to obtain amides **4** by amonolysis of the corresponding ester because of low reactivity of the compounds mentioned.

Synthesis of nitriles **5** by dehydratation of the proper amides **4** with phosphorous-oxychloride in dichlormethane was not successful in all cases. The fact mentioned was caused by low yields and problems with isolation of the target compound from the reaction mixture. Thus, nitriles 5 were synthesized by interaction of potassium thiolates with chloroacetonitrile in propanol-2.

We noted that thions **1** were also alkylated by chloroacetic acid and their derivatives in propanol-2 in the presence of sodium hydroxide. The reaction easily flows for 1-1.5 h., elongation of the process did not lead to the increase of the yields.

The purity of the compounds synthesized was confirmed by LC-MS, the structure by elemental analysis, ^{1}H and ^{13}C NMR-spectrometry.

In LC-MS spectra of compounds **3, 4** and **5** in most cases the positive ions [M+1] and [M+3] were observed, the m/z values of the ions mentioned corresponded to the molecular weight of the target compounds. In LC-MS spectra acids **3.2-3.4** we have found the signals, which according to their m/z value belong to the fragmentary ion $[M–CH_2COOH]^+$, and it additionally confirms the structure of the acids synthesized.

In ^{1}H NMR-spectra of compounds **3**, **4** and the singlet signal of the SCH_2-group at 4.33-3.89 ppm, the chemical shift of the signal was caused by the nature of the substituent at the carbonyl group. The signal of the proton mentioned was observed in the lower field of ^{1}H-NMR of the corresponding nitriles **5** (4.33-4.32 ppm). The pro-

Table

The antimicrobial and antifungal activity of the compounds synthesized

Comp. No.	R*	R_1	R_2	R_3	E. coli		S. aureus		P. aerugenosa		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
3.3	4-(Me)C_6H_4	H	H	H	100	100	25	100	50	100	50	50
3.4	3,4-$(Me)_2C_6H_3$	H	H	H	100	200	12.5	100	50	100	50	100
3.5	4-(Et)C_6H_4	H	H	H	100	200	12.5	50	100	200	50	100
3.6	4-(*i*-Pr)-C_6H_4	H	H	H	100	200	12.5	25	100	100	50	100
3.14	4-F-C_6H_4	H	H	Br	200	200	12.5	50	100	200	100	100
4.1	Me	H	H	H	50	100	100	200	200	200	25	100
4.5	4-(Et)C_6H_4	H	H	H	100	100	25	100	100	200	50	100
4.6	4-(tert-Bu)C_6H_4	H	H	H	100	200	25	100	100	200	50	100
4.7	4-(MeO)C_6H_4	H	H	H	100	200	25	25	100	200	25	50
4.8	4-(EtO)C_6H_5	H	H	H	100	100	12.5	50	100	200	50	100
4.11	Ph	Me	H	H	100	100	50	100	50	100	25	100
4.12	4-F-C_6H_4	Br	H	H	100	100	50	200	100	200	25	50
4.13	Ph	H	F	H	100	200	12.5	100	100	200	12.5	12.5
4.14	4-(MeO)C_6H_4	H	F	H	200	200	12.5	25	200	200	12.5	25
4.16	4-FC_6H_5	H	F	H	100	200	25	50	200	200	25	50
4.26	4-F-C_6H_4	H	H	Br	50	100	100	200	200	200	25	100
5.10	Ph	H	F	H	100	200	50	100	100	200	12.5	25
5.11	4-(MeO)C_6H_4	H	F	H	100	200	50	100	100	200	25	50
5.13	Ph	H	H	Cl	25	100	100	200	50	100	50	100
5.14	4-MeC_6H_4	H	H	Cl	25	100	100	200	50	100	50	100
Nitrofural					1.5	–	6.25	–	6.25	–	25.0	–
Trimethoprim					50	50	31.2	62.5	62.5	125	62.5	125
Ketoconazole					–	–	–	–	–	–	25	–

* – compounds **3.1**, **3.2**, **3.7**-**3.13**, **4.2**-**4.4**, **4.9**, **4.10**, **4.15**, **4.17**-**4.25**, **5.1**-**5.9**, **5.15**-**5.19** exhibit the antibacterial activity ≤50 μg /ml.

ton of the carboxylic group of acids was observed at 13.92-12.90 ppm. Protons of the primary amide group of compounds **4** as result of the C=O group effect were observed as two non-equivalent one-proton singlets at 7.30-7.08 ppm and 7.58-7.53 ppm. For the secondary amides **4** the amide protons were observed as a singlet at 10.59-10.00 ppm (for **4.9**, **4.16**, **4.24**, **4.25**) or 8.82-8.5 ppm **(4.19**, **4.21**, **4.22).** Compounds **(4.19**, **4.21, 4.22**) were also characterized by a two-proton doublet of the NCH_3 fragment in ^{1}HNMR spectra. Aromatic protons of the non-substituted triazinoquinazoline cycle of compounds **3, 4, 5** formed systems with two one-proton doublets H-8 and H-11 and two one-proton triplets H-9 and H-10. Signals of aromatic protons of the *para*-substituted phenyl moiety in position 3 of compounds **3**, **4** and **5** were observed as the A_2B_2-system consisting of two-proton doublets (H-3, H-5 and H-2, H-6), at the same time, signals of the unsubstituted one were observed as a multiplet (H-3, H-4, H-5) and a two-proton doublet.

Signals of sp^3-hybrid carbons belonging to CH_3 (18.19 ppm), SCH_2 (34.21-38.94 ppm), CH_3O (55.94 ppm) were observed in the high field of ^{13}C NMR-spectra of some compounds. According to^{13}C NMR spectra the most deshielded carbons were located in the -COOH-group (170.04-170.08 ppm) and positions 2 and 6 of [1,2,4]triazino[2,3-*c*]quinazoline system.

2. Antimicrobial and antifungal activities

The antimicrobial assay has shown that the compounds synthesized exhibit the antibacterial and antifungal activity against the strains studied. Thus, 2-[(3-methyl-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids (**3.1**, MIC 50-200 μg/ml) exhibit a moderate action against *E.coli, S. aureus* and *P. aeruginosa*, but a high activity against *C. albicans* (MIK 50 μg/ml, Table). Chemical modification of compound **3.1** via changing the methyl group in position 3 into the phenyl moiety (**3.2**) did not lead to the activity increase. Introduction of fluorine (**3.9**) and alkoxy groups (**3.7, 3.8**) in the phenyl moiety also did not lead to the increase of the antibacterial and antifungal activity. At the same time 4-alkylphenyl derivatives (**3.3-3.6**) exhibit a high inhibiting effect against *S. aureus* (MIC 12.5-25 μg/ml). We noticed that the increase in activity was observed in case of introduction of the additional methyl group (compound **3.4**), elongation (**3.5**) and branching (**3.6**) of the alkyl group in the phenyl substituent (Table). Introduction of the addition substituent (fluoro-, chloro-, bromo- and methyl group) in position 8, 9, 10 did not cause essential changes of the antimicrobial and antifungal activity. Only compound **3.14** containing bromine in position 10 and (4-fluoro)phenyl in position 3 exhibits a high activity against *S. aureus* (MIC 12.5 μg/ml).

Following modification of the carboxylic group (compounds **3.1-3.14**) in the amide fragment (**4.1-4.26**) resulted in increasing the antibacterial and antifungal activity against the strains studied. We noticed that some amides (**4.1**, **4.3**, **4.4**, **4.9**, **4.15**, **4.17**. **4.18**, **4.20**, **4.21**, **4.26**) unlike acids (**3.1-3.15**) exhibited the antibacterial activity against *E. coli* (MIC 50 μg/ml) at the same level as trimetoprim (MIC 50 μg/ml). Moreover, amides **4.1-4.26** showed a high antifungal action against *C. albicans* (MIC 12.5-50 μg/ml) exceeding the activity of trimetoprim (MIC 62.5 μg/ml) and was comparable to nitrofural (MIC 25.0 μg/ml, compounds **4.1**, **4.7**, **4.11**, **4.14**, **4.16**, **4.26**). A considerable attention as to antifungal agents should be paid to compounds **4.13** and **4.14** (MIC 12.5 μg/ml) exceeding the activity of ketoconazol (MIC 25 μg/ml).

We noted that as in case of acids amides **4.1-4.26** were more active against *S. aureus* (MIC 12.5-100 μg/ml). The modification of compound **4.1** was conducted via changing the methyl group in position 3 of the triazinoquinazoline system into phenyl (**4.2**), 4-alkylphenyl (**4.3**, **4.5-4.6**), 3,4-dimethylphenyl (**4.4**) and 4-alkoxyphenil (**4.7**, **4.8**) results in the activity increase. Unlike acids (**3.11**, **3.12**), amides (**4.13-4.16**) containing a fluorine atom in position 9 were more active against *S. aureus*. Substitution of fluorine (**4.13-4.16)** into bromine (**4.17**) caused insignificant decrease in activity (MIC 50 μg/ml). The complete loss of activity was observed in case of translocation of bromine from 9 to 10 position (compounds **4.22**, **4.23**, **4.26**; MIC 50-100 μg/ml) and introduction of chlorine (**4.19** MIC 50 μg/ml). Introduction of the N-substituted amide group (compounds **4.9**, **4.15**, **4.18**, **4.20**, **4.21**, **4.25**, **4.26**) irrespective of the substituent and its location in the molecule did not affect or cause insignificant decrease of action against *S. aureus*.

Nitriles (**5.1-5.19)** unlike acids (**3.1-3.15**) and amides (**4.1-4.26**) were active against *P. aeruginosa* (MIC 50-100 μg/ml). Among all the compounds studied only compounds **5.13** and **5.14** revealed a high antimicrobial action against *E. coli* (MIC 25 μg/ml). At the same time the inhibitory activity of nitriles (**5.1-5.19**) against *S. aureus* comparing to acids and amides were insignificant (MIC 50-200 μg/ml). Nitriles (**5.1-5.19**) also exhibited a high inhibitory action against *C. albicans* (MIC 12.5-100 μg/ml). Among the compounds studied our attention was focused on compound **5.10** (MIC 12.5 μg/ml) exceeding ketoconazole (MIC 25 μg/ml) in its activity.

Thus, the antimicrobial and antifungal activity of 2-[8-R_1-9-R_2-10-R_3-3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids studied and their derivatives was caused by the nature of substituents in position 6, substituents of the phenyl moiety in position and substituents in positions 8, 9, 10 of the triazinoquinazoline system. We have found that the presence of 4-alkylphenyl (compounds **3.3-3.6**, **4.3-4.6**; MIC 12.5-25 μg/ml), 4-alkoxyphenyl (**4.7**, **4.8**; MIC 12.5-50 μg/ml) substituents in position 3 and fluorine in position 9 **4.13-4.16**; MIC 12.5-25 μg/ml) contributes positively to the high activity against *S. aureus*, the presence of fluorine in position 9 also promotes the activity against *C. albicans* (**4.13**, **4.14**; MIC 12.5 μg/ml).

CONCLUSIONS

In the present paper 50 new derivatives of 2-[8-R_1-9-R_2-10-R_3-3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetic acids have been described. The compounds synthesized have been tested for the antimicrobial and antifungal activity using standard test cultures: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 885-653. It has been shown that the compounds synthesized exhibit a high antimicrobial activity against *St. aureus* (compounds **3.3-3.6, 4.3-4.6, 4.7, 4.8, 4.13-4.16**; MIC 12,5-25 μg/ml) and *C. albicans* (compounds **4.13, 4.14**; MIC 12,5 μg/ml). The "structure-activity" relationship has been discussed.

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СИНТЕЗ ТА МОДИФІКАЦІЯ 2-[(8-R_1-9-R_2-10-R_3-3-R-2-ОКСО-2*H*-[1,2,4]ТРИАЗИНО[2,3-*c*] ХІНАЗОЛІН-6-ІЛ)ТІО]ОЦТОВИХ КИСЛОТ, СПРЯМОВАНІ НА ПОШУК СПОЛУК З АНТИБАКТЕРІАЛЬНОЮ ТА ПРОТИГРИБКОВОЮ ДІЄЮ

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Ключові слова: *синтез; хіназоліни; триазини; антибактеріальна активність; протигрибкова активність*

В представленій роботі описано синтез 50 нових похідних 2-[8-R_1-9-R_2-10-R_3-3-R-2-оксо-2H-[1,2,4]триазино[2,3-c]хіназолін-6-іл)тіо]оцтових кислот. Показано, що алкілування калій 8-R_1-9-R_2-10-R_3-3-R-2-оксо-2H-[1,2,4]триазино[2,3-c]хіназолін-6-тіолатів хлороцтовою кислотою, хлорацетамідом, N-R_4-хлорацетамідами та хлорацетонітрилом веде до утворення відповідних 2-[8-R_1-9-R_2-10-R_3-3-R-2-оксо-2H-[1,2,4]триазино[2,3-схіназолін-6-іл)тіо]оцтових кислот, їх амідів та нітрилів. Для відповідних кислот та нітрилів були опрацьовані альтернативні синтетичні підходи. Також були обговорені обмеження у синтезі цільових сполук. Так, було показано, що аміди 2-[8-R_1-9-R_2-10-R_3-3-R-2-оксо-2H-[1,2,4]триазино[2,3-c]хіназолін-6-іл)тіо]оцтових кислот не можуть бути одержані амонолізом відповідних естерів внаслідок низької реакційної здатності останніх. Заявлено, що синтез нітрилів дегідратацією відповідних амідів хлорокисом фосфору у дихлорометані був успішним не в усіх випадках. Зазначений факт обумовлено вкрай низькими виходами цільових сполук, що пов'язано зі значними проблемами при їх виділенні з реакційної суміші. Структуру синтезованих сполук було визначено за допомогою комплексу сучасних фізико-хімічних методів (^{1}H, ^{13}C NMR, LC-MS-спектрами). Синтезовані сполуки були випробувані на антимікробну та протигрибкову дію з використанням стандартних тест-культур: Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 та Candida albicans ATCC 885-653. Показано, що сполуки 3.3-3.6, 4.3-4.6, 4.7, 4.8, 4.13-4.16 проявляють виражену активність по відношенню до St. aureus (MIC 12,5-25 μg/ml), а сполуки 4.13, 4.14 також по відношенню до C. albicans (MIC 12,5 μg/ml). В рамках статті обговорено взаємозв'язок «структура-біологічна дія».

СИНТЕЗ И МОДИФИКАЦИЯ 2-[(8-R_1-9-R_2-10-R_3-3-R-2-ОКСО-2*H*-[1,2,4]ТРИАЗИНО[2,3-*c*] ХИНАЗОЛИН-6-ИЛ)ТИО]УКСУСНЫХ КИСЛОТ, НАПРАВЛЕННЫЕ НА ПОИСК СОЕДИНЕНИЙ С АНТИБАКТЕРИАЛЬНЫМ И ПРОТИВОГРИБКОВЫМ ДЕЙСТВИЕМ

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Ключевые слова: *синтез; хиназолины; триазины; антибактериальная активность; противогрибковая активность*

В представленной работе описан синтез 50 новых производных 2-[8-R_1-9-R_2-10-R_3-3-R-2-оксо-2H-[1,2,4]триазино[2,3-c]хиназолин-6-ил)тио]уксусных кислот. Показано, что алкилирование калий 8-R_1-9-R_2-10-R_3-3-R-2-оксо-2H-[1,2,4]триазино[2,3-c]хиназолин-6-тиолатов хлоруксусной кислотой, хлорацетамидом, N-R_4-хлорацетамидами и хлорацетонитрилом приводит к образованию соответствующих 2-[8-R_1-9-R_2-10-R_3-3-R-2-оксо-2H-[1,2,4]триазино[2,3-c]хиназолин-6-ил)тио]уксусных кислот, их амидов и нитрилов. Для соответствующих кислот и нитрилов были разработаны альтернативные синтетические подходы. Также были обговорены ограничения в синтезе целевых соединений. Так было показано, что амиды 2-[8-R_1-9-R_2-10-R_3-3-R-2-оксо-2H-[1,2,4]триазино[2,3-c]хиназолин-6-ил)тио]уксусных кислот не могут быть получены амонолизом соответствующих эфиров ввиду низкой реакционной активности последних. Заявлено, что синтез нитрилов дегидратацией соответствующих амидов хлорокисью фосфора в дихлорметане был успешен не во всех случаях. Данный факт был обусловлен крайне низкими выходами целевых соединений вследствие значительных проблем при их выделении из реакционных смесей. Структура синтезированных соединений подтверждена с помощью современных физико-химических методов (^{1}H, ^{13}C NMR, LC-MS-спектры). Синтезированные соединения были исследованы на противомикробную и противогрибковую активность с использованием стандартных тест культур: Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 и Candida albicans ATCC 885-653. Показано, что соединения 3.3-3.6, 4.3-4.6, 4.7, 4.8, 4.13-4.16 проявляют выраженную активность по отношению к St. aureus (MIC 12,5-25 μg/ml), а соединения 4.13, 4.14 также по отношению к C. albicans (MIC 12,5 μg/ml). Также в рамках статьи обсуждена взаимосвязь «структура-биологическое действие».

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