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SYNTHESIS AND BIOLOGICAL ACTIVITY OF A NOVEL SERIES OF 6,7-DIMETHOXYQUINAZOLINE-2,4(1*H*,3*H*)-DIONE DERIVATIVES

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The heterocyclic compounds are very important part of medicinal chemistry, among them it is worth to pay attention on derivatives of quinazoline. They have a broad spectrum of pharmacological activities like anticancer (1–3), anti-HIV (3), antitubercular (4), anti-inflammatory (5), anticonvulsant (6) and antihypertensive activity (7). The most recognized derivatives among quinazolines with antihypertensive activity are bunazosin or alfuzosin. Moreover, it was also reported that quinazolines have an interesting antimicrobial activity against different species of pathogenic G-positive bacteria, G-negative bacteria and fungi (8–12). Generally, it seems that quinazoline combined with different type of aryl, heteryl as well as alkyl substituents showed awide range of pharmacological applications.

In our earlier work, we also showed that compounds containing short amine fragments exhibit antibacterial and antifungal activity (13, 14). Therefore, it was assumed that compounds having quinazoline residue in the molecule condensed with short alkyl amine may possess some interesting biological activity. In light of this information, we have decided to continue the study on the microbial activity of imide moiety condensed with short amine. This work represents a continuation of our systematic studies of compounds condensed with short alkylamine as anticancer and antibacterial agents.

The starting material – 6,7-dimethoxyquinazoline-2,4(1*H*,3*H*)-dione, was converted into N,N'-dialkylamine derivatives by using appropriate chloroalkylamines. Scheme 1 explains the method of preparation of derivatives mentioned in the title of this work. Next, all derivatives were tested for antimicrobial activity against a selection of G-positive, G-negative bacteria, yeasts and also the chosen compounds (**II**, **IV**) were tested for their cytotoxic properties in K562, HeLa and normal cells. The results are presented in Table 1.

Table 1. The IC_{50} values calculated from the dose for compounds **II** and **IV**.

	HeLa	K562	HUVEC
Compound	IC_{50} 48 h	IC_{50} 48 h	IC_{50} 48 h
II	> 1 mM	400 μM	200 μM
IV	600 μM	300 μM	100 μM

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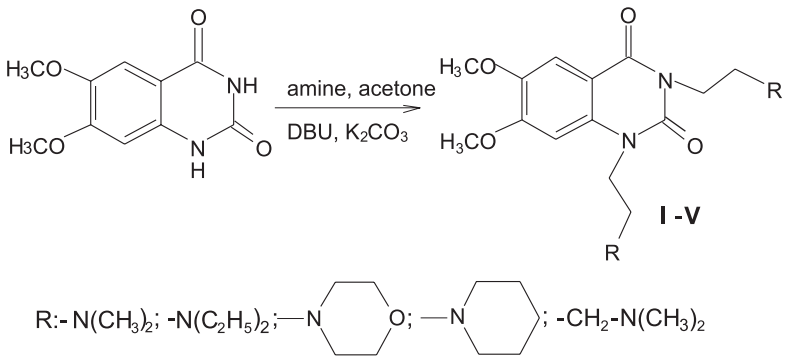

Scheme 1. Method of preparation of compounds **I**–**V**

EXPERIMENTAL

Chemistry

Melting points were determined in capillaries in an Electrothermal 9100 apparatus and were uncorrected. The proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded in DMSO-d_6 on a Bruker VMNRS300 instrument operating at 300 MHz. Chemical shift values are expressed in ppm (parts per million) in relation to tetramethylsilane as an internal standard and coupling constants *J* are given in Hz. Mass spectral ESI (Electrospray Ionization) measurements were carried out on a Mariner Perspective – Biosystem instrument with TOF detector. The spectra were obtained in the positive ion mode with a declustering potential 140–300 V. Chromatographic columns were filled with Merck Kieselgel 0.05–0.2 mm reinst (70–325 mesh ASTM) silica gel. Reactions were monitored by TLC on silica gel (plates with 254 nm fluorescent indicator, layer thickness 0.2 mm, Kieselgel G, Merck), using mixture chloroform/methanol 9.8:0.2 or 9.5:0.5, v/v as eluents.

General procedure of preparing N,N'-dialkylamino derivatives (**1**–**5**)

The imide (0.01 mol) was dissolved in acetone (30 mL), then powdered anhydrous K_2CO_3 (0.01 mol) and catalytic amount of 98% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and an appropriate chloroalkylamine (0.03 mol) were added. The reaction mixture was heated for 8–10 h. After the reaction completion, the inorganic residue was filtered off and the solvent was evaporated. The obtained compound was purified by column chromatography using pure chloroform or mixture chloroform/methanol 50:0.2, v/v as eluents.

6,7-Dimethoxy-1,3-bis[2-(dimethylamino)ethyl]quinazoline-2,4(1*H*,3*H*)-dione (**I**)

Yield: 68%; m.p. 79.5–81.6°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.18 (6H, s, -CH_3), 2.23 (6H, s, -CH_3), 2.44 (4H, m, -CH_2-), 3.82 (3H, s, -OCH_3), 3.94 (3H, s, -OCH_3), 4.04 (2H, *t*, *J* = 6.9 Hz, -CH_2-), 4.22 (2H, t, *J* = 6.9 Hz, -CH_2-), 6.90 (1H, s, Ar-H), 7.42 (1H, *s*, Ar-H); MS (m/z): 100% = 365.1, 8% = 366.2 [L+H$^+$].

6,7-Dimethoxy-1,3-bis[2-(diethylamino)ethyl]quinazoline-2,4(1*H*,3*H*)-dione (**II**)

Yield: 72%; m.p. 75.3–76.8°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 0.91 (12H, m, -CH_3), 2.60 (12H, m, -CH_2-), 3.81 (3H, s, -OCH_3), 3.93 (3H, s, -OCH_3), 3.99 (2H, m, -CH_2-), 4.18 (2H, t, *J* = 6.9 Hz, -CH_2-), 6.91 (1H, s, Ar-H), 7.41 (1H, s, Ar-H); MS (m/z): 100% = 421.2, 5% = 422.2 [L+H$^+$].

6,7-Dimethoxy-1,3-bis[2-(morpholin-4-yl)ethyl]quinazoline-2,4(1*H*,3*H*)-dione (**III**)

Yield: 75%; m.p. 200–204°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.43 (6H, m, H-morpholine), 2.54 (4H, m, -CH_2-), 3.52 (10H, m, H-morpholine), 3.82 (3H, s, -OCH_3), 3.95 (3H, s, -OCH_3), 4.07 (2H, t, *J* = 6.9Hz, -CH_2-), 4.27 (2H, t, *J* = 6.9 Hz, -CH_2-), 6.91 (1H, s, Ar-H), 7.42 (1H, s, Ar-H); MS (m/z): 100% = 449.2 [L+H$^+$], 20% = 471.1 [L+Na$^+$].

6,7-Dimethoxy-1,3-bis[2-(piperidin-1-yl)ethyl]quinazoline-2,4(1*H*,3*H*)-dione (**IV**)

Yield: 69%; m.p. 183–184.6°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.35 (4H, m, H-piperidine), 1.44 (10H, m, H-piperidine), 2.41 (10H, m, -CH_2-, H-piperidine), 3.81 (3H, s, -OCH_3), 3.94 (3H, s, -OCH_3), 4.04 (2H, t, *J* = 7.0 Hz, -CH_2-), 4.23 (2H,

t, J = 6.9Hz, -CH_2-), 6.91 (1H, s, Ar-H), 7.41 (1H, s, Ar-H); MS (m/z): 100% = 445.1, 11% = 446.1 [L+H^+].

6,7-Dimethoxy-1,3-bis[3-(dimethylamino)propyl]quinazoline-2,4(1*H*,3*H*)-dione (V)

Yield: 72%; m.p. 97–98°C; ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.71(4H, m, -CH_2-), 2.11 (6H, *s*, -CH_3), 2.13 (6H, *s*, -CH_3), 2.27 (4H, *m*, -CH_2-), 3.82 (3H, *s*, -OCH_3), 3.95 (5H, m, -OCH_3, -CH_2-), 4.14 (2H, , J = 7.2Hz, -CH_2-), 6.94 (1H, s, Ar-H), 7.43 (1H, s, Ar-H); MS (m/z): 100% = 393.0 [L+H^+], 10% = 415.1 [L+Na^+].

Microbiology

Organisms

The standard strains of *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 14053 and one clinical isolate *S. maltophilia* CO2275 were used.

Screening for the antimicrobial activity

The method according to CLSI (Clinical and Laboratory Standards Institute) directives was applied (15). Compounds **I–V** were tested for their bacteriostatic activity at the high concentrations (512 mg/L).

The tested substances were dissolved in DMSO and then the solutions were added to brain heart infusion broth (BHI-B) medium to the final concentration 512 mg/L.

The bacteria were cultured on the plates with BHI agar (BHI-A) medium supplemented with 7% horse blood, at temperature 35–37°C, in an aerobic atmosphere, for 18–24 h. The fungal strain was cultured in the Sabouraud agar (SA), at the same temperature and atmosphere, but for at least 24 h. The cultures, which were in mid-logarithmic phase of growth, were suspended in 0,9 % NaCl solution to obtain 0,5 Mac Farland's optical density. 1.0–9.0 × 10^5 cells (0.1 mL of the prepared suspension) were added to sample tubes with 2 mL of BHI-B broth medium containing the tested substances. Samples were incubated at 35–37°C for 24–48 h. If after 48 h the growth was absent, the substance was noticed as potentially possessing antimicrobial activity.

In all experiments strains, vitality controls and DMSO antimicrobial activity controls in the applied concentrations were performed.

Cells and cytotoxicity assay

Human umbilical vein endothelial cells (HUVEC) were isolated from freshly collected umbilical cords and cultured in plastic dishes coated with gelatin, in RPMI 1640 medium supplemented with 20% FBS, 90 U/mL heparin, 150 µg/mL ECGF (Roche Diagnostics, Mannheim, Germany) and antibiotics (100 µg/mL streptomycin and 100 U/mL penicillin). 10 × 10^3 cells were seeded on each well on 96-well plate (Nunc).

The HeLa (human cervix carcinoma) and K562 (leukemia), cells were cultured in RPMI 1640 medium supplemented with antibiotics and 10% fetal calf serum, in a 5% CO_2 – 95% air atmosphere. Cells (7 × 10^3) were seeded on each well on 96-well plate (Nunc). Forty eight hours later, cells were exposed to the test compounds. Stock solutions (100 mM) of test compounds were freshly prepared in DMSO. The final concentrations of the compounds tested in the cell cultures were: 1, 1 × 10^{-2}, 1 × 10^{-4} and 1 × 10^{-6} mM. The concentration of DMSO in the cell culture medium was 1%.

The values of IC_{50} (the concentration of test compound required to reduce the cell survival fraction to 50% of the control) were calculated from dose-response curves and used as a measure of cellular sensitivity to a given treatment.

The cytotoxicity of all compounds was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma, St. Louis, MO] assay as described (16). Briefly, after 24 or 48 h of incubation with drugs, the cells were treated with the MTT reagent and incubation was continued for 2 h. MTT-formazan crystals were dissolved in 20% SDS and 50% DMF at pH 4.7 and absorbance was read at 570 and 650 nm on an ELISA-PLATE READER (FLUOstar Omega). As a control (100% viability), we used cells grown in the presence of vehicle (1% DMSO) only.

RESULTS AND DISCUSSION

We have synthesized a series of five derivatives of 6,7-dimethoxyquinazoline-2,4(1*H*,3*H*)-dione. All compounds were tested for their antimicrobial activity against four microbial species: *Staphylococcus aureus, Escherichia coli, Stenotrophomonas maltophilia and Candida albicans* and some (**II**, **IV**) were tested for their cytotoxic properties in K562 (leukemia) and HeLa (cervix carcinoma) cells.

Our earlier studies showed that antimicrobial activity depends on the amine type (13, 14). We could observe an increase or a decrease of this activity. The stronger and broader spectra of activity were obtained for compounds containing short-amine or cyclic-amine fragments. Surprising are the results of this study because among examined com-

pounds none displayed any bacteriostatic activity. They did not inhibit bacterial growth, even at high concentrations (512 mg/L) despite of the fact that in their structures we can find two active fragments: quinazoline-2,4(1*H*,3*H*)-dione and short-amine, what we mentioned in the introduction.

In the cytotoxic screening studies, two human cancer cell lines (K562 and HeLa) and normal endothelial cells (HUVEC) were used. Cells treated with 1% DMSO served as the control (100% viability in the MTT assay). The viability of cells was determined at four different drug concentrations: 1, 1×10^{-2}, 1×10^{-4} and 1×10^{-6} mM. Our results indicate that after 48 h incubation compounds **II** and **IV** showed limited toxicity toward K562 (leukemia) and HUVEC cells with IC_{50} values in the range of 100 to 400 μM (Table 1). These compounds were the least toxic for HeLa (cervix carcinoma) cells, which is evidenced by the fact that for compound **II** we were unable to calculate IC_{50} value. Although quinazoline derivatives are reported as possessing anticancer activity (1–3), our study shows that quinazoline condensed with short alkylamine chains (compounds **II** and **IV**) have very limited anti-cancer activity. Moreover, as evidenced by calculated IC_{50} values (Table 1) these compounds seem to be more toxic toward normal endothelial cells than toward cancer cells, which further limits their potential anticancer applications.

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