PRIMARY ANTIMICROBIAL SCREENING OF NOVEL [1,2,4]TRIAZOLO[4,3-a]QUINAZOLIN-5(4H)-ONE DERIVATIVES

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Aim. The aim of the given study was to conduct primary antimicrobial screening of novel [1,2,4]triazolo[4,3-a]quinazolin-5(4H)-one derivatives.

Methods. The set of 169 novel [1,2,4]triazolo[4,3-a]quinazolin-5(4H)-one derivatives has been tested for activity against 5 bacteria: Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Staphylococcus aureus, and 2 fungi: Candida albicans and Cryptococcus neoformans. Primary antimicrobial screening has been conducted by whole cell growth inhibition assays, using the provided samples at a single concentration. Samples were tested in water – 0.3% DMSO solutions with final sample concentrations 32 µg/ml (70–80 µMol).

Results. [1,2,4]TriaZol[4,3-a]quinazolin-5(4H)-ones 5[1], 7[1], 7[2], 7[3] showed more than 80% inhibition of Acinetobacter baumannii growth and compounds 7[4] showed more than 80% inhibition of growth fungi Cryptococcus neoformans.

Conclusions. For the first time conducted antimicrobial screening of novel [1,2,4]triazolo[4,3-a]quinazolin-5(4H)-ones showed that compounds, which had no amide group exhibited no antimicrobial activity, but several [1,2,4]triazolo[4,3-a]quinazolin-5(4H)-one derivatives containing amide group attached by carbon or sulfur-carbon chain possess antimicrobial activity against Acinetobacter baumannii or fungi Cryptococcus neoformans.

Keywords: [1,2,4]triazolo[4,3-a]quinazolin-5(4H)-one, antimicrobial activity Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Cryptococcus neoformans


Перший скрінінг на антимікробну активність було проведено тестуванням водного масиву на подавлення росту клітин, за умов однакової концентрації сполук. Розчин сполук було тестовано у водних з 0.3% DMSO розчинах при остаточній концентрації сполук 32 µMol (70–80 µMol).
1. Introduction
The infection treatment was actual task of medicine both ancient and modern times. A lot of remedies were directed against infectious diseases. But massive use of antimicrobial agents leads to appear microbial resistance to popular drugs.

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues
The growth of microbial resistance to drugs is serious problem in the infection treatment. The main solution to this problem is the search for new effective antimicrobial agents. Usually, the screening of assays of new derivatives potentially biologically active compounds for antimicrobial activity is efficient way to find new antimicrobial substances.

Derivatives of 1,2,4-triazolo[4,3-a]quinazolin-5(4H)-one, which are representatives of the important class of condensed heterocycles possessing wide range of the biological activity, attract particular interest in development of innovative drug substances.

3. Analysis of recent studies and publications in which a solution of the problem and which draws on the author

4. Allocation of unsolved parts of the general problem, which is dedicated to the article
Only a small part of the vast array of possible 1,2,4-triazolo[4,3-a]quinazolin-5(4H)-one derivatives, namely, 1-alkyl-3-aryl[1,2,4]triazolo[4,3-a]quinazolin-5(4H)-ones have been systematically screened for antimicrobial activity [11, 12].

5. Formulation of goals (tasks) of article
The aim of the given study was to conduct primary antimicrobial screening of novel 1,2,4-triazolo[4,3-a]quinazolin-5(4H)-one derivatives, namely, 1-aryl-3-alkyl[1,2,4]triazolo[4,3-a]quinazolin-5(4H)-ones and 1,2,4-triazolo[4,3-a]quinazolin-5(4H)-ones containing amide group.

6. Statement of the basic material of the study (methods and objects) with the justification of the results
We used formerly described [16] 2-hydrazinoquinazolin-4(3H)-ones 1 as starting materials for 1,2,4-triazolo[4,3-a]quinazolin-5(4H)-ones synthesis (Fig. 1).

1-Aryl-3-alkyl[1,2,4]triazolo[4,3-a]quinazolin-5(4H)-ones 2 have been synthesized by reaction of 2-hydrazinoquinazolin-4(3H)-ones 1 with aromatic aldehydes followed by oxidation in the presence of FeCl₃ according [17].

Amides of [4-substituted-4,5-dihydro-5-oxo[1,2,4]triazolo[4,3-a]quinazolin-1-yl]thioacetic acid 5 have been obtained according [15] by condensation of hydrazines 1 with CS₂ resulted in 1-thioxo-2,4-dihydro[1,2,4]triazolo[4,3-a]quinazolin-5(4H)-ones 3 formation. Consequent alkylation by chloroacetic acid amides 4 lead to [1,2,4]triazolo[4,3-a]quinazolin-5(4H)-ones 5, which contain sulfur-carbon chain.

Amides of 3-(4-substituted-5-oxo-4,5-dihydro[1,2,4]triazolo[4,3-a]quinazolin-1-yl)propanoic acid 7 have been obtained according [15, 18] by condensation of hydrazine 1 with succinic anhydride, that produced 3-(4-substituted-5-oxo-4,5-dihydro[1,2,4]triazolo [4,3-a]quinazolin-1-yl)propanoic acids 6. For amide formation we used activation of carboxylic group in obtained acid 6 via getting intermediate imidazolyl amide by carbonyldimidazole (CDI) in anhydrous dioxane with subsequent reaction with corresponding amine by reflux up to 2 hours.


The structures of obtained compounds have been confirmed by the \(^1\)H NMR spectroscopy data. Formation of the [1,2,4]triazolo[4,3-a]quinazolin-5(4H)-one condensed system led to shift of H-6 protons signals to 8.26–9.22 ppm, that is in good correlation with the known data [19]. \(^1\)H NMR-spectra were recorded on Varian WXR-400 (200 MHz) spectrometer in DMSO-d6 solution with TMS as internal standard, chemical shifts are reported in ppm. Melting points were measured with a Buchi B-520 melting point apparatus. Elemental analysis was performed on Euro EA-3000 apparatus.

4-Benzyl-N-isopropyl-1,5-dioxo-1,2,4,5-tetrahydro[1,2,4]triazolo[4,3-a]quinazoline-8-carboxamide 9. Reflux the solution of 0.70 g (0.002 mol) 3-benzy1-2-hydrazino-N-isopropyl-4-oxo-3,4-dihydroquinazoline-7-carboxamide 8 and 0.39 g (0.0024 mol) of CDI in anhydrous DMF (5 ml) during 6 hours. After cooling, dilute the reaction mixture with H\(_2\)O (20 ml). Filter the precipitate formed, wash it with i-propanol (10 ml) and recrystallize from mixture of DMF (5 ml) and i-propanol (10 ml). Yields 0.35 g (71 %), 4-Benzyl-2-[2-((tert-butylamino)-2-oxoethyl]-N-isopropyl-1,5-dioxo-1,2,4,5-tetrahydro[1,2,4]triazolo[4,3-a]quinazoline-8-carboxamide 11. Dissolve 0.38 g (0.001 mol) of 4-benzy1-N-isopropyl-1,5-dioxo-1,2,4,5-tetrahydro[1,2,4]triazolo[4,3-a]quinazoline-8-carboxamide 9 in 5 ml of DMF. Add 0.21 g (0.002 mol) of powder of anhydrous Na\(_2\)CO\(_3\) with stirring. Add 0.18 g (0.0012 mol) of N-(tert-butyl)-2-chloroacetamide 10. Heat the reaction mixture with stirring at 90 \(^\circ\)C for 2 hours. After cooling, dilute the reaction mixture with H\(_2\)O (20 ml). Filter the precipitate formed, wash it with i-propanol (10 ml) and recrystallize from mixture of DMF (5 ml) and i-propanol (10 ml). Yields 0.35 g (71 %).
white solids, m.p. 294-296 °C. 1H NMR (δ, ppm, J, Hz): 1.08 (9H, d, J=7.0, 3CH_{3}); 1.16 (6H, d, J=7.0, 2CH_{2}); 4.02-4.12 (1H, m, CH(CH_{3})_{2}); 5.20 (2H, s, CH_{2}); 5.20 (2H, s, CH_{2}); 7.24-7.36 (3H, m, H-3,4,5 Bn); 7.48 (2H, d, J=7.8, H-2,6 Bn); 7.86 (1H, dd, J_{6}=7.8, J_{7}=2.0, H-7); 8.05 (1H, d, J=7.0, CONH); 8.17 (1H, d, J_{6}=7.8, H-6); 8.57 (1H, d, J=7.0, CONH); 9.00 (1H, d, J_{7}=2.0, H-9). Found: %: C 63.54; H 6.18; N 17.08. C_{37}H_{60}N_{5}O_{8}. Calculated, %: C 63.66; H 6.16; N 17.13.


This set of compounds has been tested for activity against 5 bacteria: Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Staphylococcus aureus, and 2 fungi: Candida albicans and Cryptococcus neoformans.

Samples of 2 mg of each compound were dissolved in 0.2 ml of DMSO that gave 10mg/ml solution in DMSO. An aliquot of each sample was diluted to 320 μg/ml in water, and plated in 384-well polypropylene plates (PP), 5 μl was plated in duplicate (n=2) into a 384-well well non-binding surface plate (NBS) for each strain or cell type assayed against. Once cells were added this gave a final compound concentration range of 32 μg/ml (70–80 μMol). Final concentration of DMSO was 0.3 %.

All bacteria were cultured in Cation-adjusted Mueller Hinton broth (CAMHB) at 37 °C overnight. The resultant mid-log phase cultures was added to each well of the compound containing plates, giving a cell density of 5 10^5 CFU/ml. All the plates were covered and incubated at 37 °C for 18 h without shaking. Inhibition of bacterial growth was determined by measuring absorbance at 600 nm using a Tecan M1000 Pro monochromator plate reader.

Fungi strains were cultured for 3 days on Yeast Extract-Peptone Dextrose (YPD) agar at 30 °C. A yeast suspension of 1 10^6 to 5 10^6 cells/ml (as determined by OD530) was prepared from five colonies. These stock suspensions were diluted with Yeast Nitrogen Base (YNB) broth to a final concentration of 2.5 10^5 CFU/ml. Then, 45 μl of the fungi suspension was added to each well of the compound-containing plates. Plates were covered and incubated at 35°C for 24 h without shaking. Growth inhibition of Candida albicans was determined measuring absorbance at 530 nm (OD530), while the growth inhibition of Cryptococcus neoformans was determined measuring the difference in absorbance between 600 and 570 nm (OD600-570), after the addition of resazurin (0.001 % final concentration) and incubation at 35 °C for additional 2 h. The absorbance was measured using a Biotek Synergy HTX plate reader.

Colistin and Vancomycin were used as positive bacterial inhibitor standards for Gramnegative and Grampositive bacteria, respectively. Fluconazole was used as a positive fungal inhibitor standard for Candida albicans and Cryptococcus neoformans.

The tests have been carried out in CO-ADD laboratory (Brisbane, Australia).
7. Conclusions
For the first time conducted antimicrobial screening of novel [1,2,4]triazolo[4,3-a]quinazolin-5(4H)-ones showed that compounds, which had no-amide group exhibited no antimicrobial activity, but several 1,2,4-triazolo[4,3-a]quinazolin-5(4H)-one derivatives containing amid group attached by carbon or sulfur-carbon chain possess antimicrobial activity against Acinetobacter baumannii and fungi Cryptococcus neoformans (Fig. 2).

References

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