ANNOTATION

Dissertation for the degree of Doctor of Philosophy (PhD) in the educational program “8D05301 – Chemistry”

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«Synthesis, structure and biological activity of new derivatives based on quinine alkaloid»

General characteristics of the work. The present research is devoted to the synthesis, structural modification, and comprehensive biological evaluation of new Cinchona alkaloid derivatives based on quinine, functionalized at the C-9 position with triazole and nitrogen-containing fragments. The study integrates methods of organic synthesis, spectroscopic analysis, in vitro and in vivo biological assays, investigations of analgesic, anti-inflammatory, cytotoxic, antimicrobial, and antibiofilm activities, as well as molecular docking, in order to establish structure–activity relationships and to identify promising antibacterial agents.

Relevance of the work. Cinchona alkaloids, and quinine in particular, belong to the class of natural compounds characterized by a unique combination of structural complexity and a broad spectrum of biological activity. Quinine, isolated from the bark of trees of the *Cinchona* genus, has long been used in medical practice as an antimalarial agent and represents a classical example of a natural alkaloid with pronounced pharmacological efficacy. In addition to its antimalarial activity, quinine and its derivatives exhibit antibacterial, antiviral, anti-inflammatory, analgesic, and antioxidant properties, which confirms their multifunctional biological potential.

The incorporation of triazole, isoxazole, isothiazole, and other nitrogen-containing fragments into the quinine structure contributes to the expansion of the chemical and biological space of its derivatives and to the formation of hybrid molecules with potentially synergistic effects. This approach is consistent with current trends in medicinal chemistry aimed at the development of multitarget molecules capable of simultaneously acting on several biological targets, including enzymatic systems and processes of bacterial biofilm formation.

Purpose of the study. The aim of this study is to synthesize new derivatives of Cinchona alkaloids based on quinine, to elucidate their molecular structures, and to investigate their biological activity.

Objectives of the study:

1. To obtain C-9 functionalized quinine derivatives through investigation of quinine O-propargylation, mesylation, and azidation reactions, as well as through the

synthesis of triazole-containing quinine derivatives via Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) based on O-propargylquinine and 9-azido-9-deoxyquinine.

2. To investigate the propargylation reaction of 9-triazolyl-substituted epi-quinine derivatives, establish the diastereoselectivity of the process, obtain (9S)- and (9R)-diastereomeric series, and implement the Cu-catalyzed three-component A^3 reaction for the introduction of various secondary amine substituents at the C-9 position of quinine.

3. To establish the molecular structures and stereochemistry of the newly synthesized derivatives using IR, ^1H and ^{13}C NMR spectroscopy (HSQC, HMBC, COSY, NOESY), mass spectrometry, and X-ray crystallographic analysis.

4. To investigate the biological activities of the synthesized derivatives, perform molecular docking studies, analyze structure–activity relationships (SAR), and identify promising compounds as potential biologically active agents.

Research Methods. A complex of complementary experimental and computational methods was employed within the framework of this dissertation research. Target compounds were synthesized using conventional organic synthesis techniques, while their purification was achieved by column and thin-layer chromatography. The identification of composition and elucidation of molecular structures were performed based on IR, UV, ^1H and ^{13}C NMR spectroscopy (HSQC, HMBC, COSY, NOESY), high-resolution mass spectrometry, X-ray crystallographic and elemental analyses, as well as high-performance liquid chromatography.

The biological activity of the synthesized compounds was investigated *in vitro* using microbiological methods, including agar diffusion, serial dilution assays, and bacterial biofilm formation models. For interpretation of experimental data and prediction of interactions between the studied molecules and biological targets, quantum-chemical calculations, molecular modeling, and molecular docking methods were applied, including studies involving the MurB enzyme. Cytotoxicity of the new quinine derivatives was evaluated using the *Artemia salina* larval bioassay, enabling preliminary toxicity screening of the investigated compounds.

Analgesic activity was studied *in vivo* using the acetic acid-induced writhing model in mice, with sodium diclofenac employed as the reference drug. Anti-inflammatory activity of the investigated compounds was evaluated *in vivo* using a model of acute exudative response (peritonitis) in outbred white rats weighing 190–210 g, with sodium diclofenac used as the reference drug.

The combination of experimental and theoretical approaches provided a comprehensive evaluation of the biological potential of the synthesized quinine derivatives.

Main statements submitted for defense

1. An efficient method for the selective introduction of functional groups at the C-9 position of the quinine alkaloid has been developed, involving O-propargylation,

mesylation, and nucleophilic substitution steps. It was established that O-propargylation of quinine in dry DMF in the presence of NaH proceeds selectively to afford O-propargylquinine in yields up to 80%, enabling its use as an effective precursor for subsequent click reactions. A method for the selective synthesis of 9-azido-9-deoxyquinine via nucleophilic substitution of the mesylate group by the azide ion in a polar aprotic solvent was developed. Optimization of the temperature regime afforded the target quinine azide in yields up to 79%, confirming the high reactivity of the intermediate. Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) was introduced as a key tool for modular modification of quinine, providing regioselective formation of the 1,2,3-triazole fragment. Using this approach, new triazole quinine derivatives were synthesized for the first time in yields up to 70%.

2. The possibility of controlled inversion of configuration at the C-9 position during O-propargylation of triazole quinine derivatives in the presence of strong bases has been established. As a result, new readily separable diastereomers were obtained in combined yields up to 70%, expanding access to stereochemically distinct derivatives and enabling investigation of the influence of configuration on biological activity. For the first time, the Cu-catalyzed three-component A^3 coupling reaction (aldehyde–alkyne–amine) was investigated for modification of C-9-substituted quinine derivatives. It was shown that this approach enables one-step introduction of secondary amine-containing fragments. As a result, the target propargylamine derivatives were obtained in yields up to 88%.

3. For the first time, the molecular structures and stereochemistry of the newly synthesized quinine derivatives were established and confirmed by IR, ^{1}H and ^{13}C NMR spectroscopy (HSQC, HMBC, COSY, NOESY), mass spectrometry, and X-ray crystallographic analysis.

4. For the first time, the biological activities of the newly synthesized derivatives were investigated, molecular docking studies were performed, structure–activity relationships (SAR) were analyzed, and corresponding regularities were established.

Characteristics of the main research results. As a result of the conducted research, efficient synthetic approaches for the selective functionalization of the quinine alkaloid at the C-9 position have been developed. Optimal conditions for O-propargylation, mesylation, and azidation reactions were established, ensuring the formation of target products in high yields. Using the Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction, a series of new 1,2,3-triazole quinine derivatives was synthesized for the first time with high regioselectivity.

The study demonstrated the possibility of controlling the configuration at the C-9 position of the quinine alkaloid; new (9R)- and (9S)-diastereomers were obtained, and the influence of stereochemistry on biological activity was established. In addition, a Cu-catalyzed three-component A^3 reaction was implemented, resulting in the regioselective formation of new derivatives containing secondary amine fragments.

Based on the chemical transformations performed, 34 quinine derivatives were synthesized, of which 27 are new compounds.

Biological investigations revealed that a number of the synthesized compounds exhibit pronounced antibacterial, antimicrobial, antibiofilm, anti-inflammatory, antioxidant, analgesic, and cytotoxic activities. Utility model patents of the Republic of Kazakhstan (№ 9011, 10114, 9770, and 8794) were granted for the most biologically active compounds.

Scientific novelty of the work

1. For the first time, efficient approaches to the selective functionalization of the quinine alkaloid at the C-9 position have been developed through O-propargylation, mesylation, and azidation steps. Based on Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reactions between O-propargylquinine and organic azides, as well as between 9-azido-9-deoxyquinine and terminal alkynes, new triazole quinine derivatives previously undescribed in the literature were synthesized. The influence of the alkyne fragment structure on reaction yields and properties of the target compounds was established.

2. For the first time, the propargylation reaction of 9-triazolyl-substituted epi-quinine derivatives has been investigated, resulting in the formation of diastereomeric pairs of (9S)- and (9R)-derivatives. It was shown that the process is accompanied by partial inversion of configuration at the C-9 position, while the diastereomeric ratio depends on the reaction conditions. In addition, a Cu-catalyzed three-component A^3 reaction (aldehyde–alkyne–amine) was implemented for the introduction of various secondary amine substituents at the C-9 position of cinchona alkaloids. It was established that this approach enables efficient and diastereoselective formation of propargylamine fragments in the quinine framework, significantly expanding the structural diversity of the obtained derivatives.

3. Based on the chemical transformations performed, 34 quinine alkaloid derivatives were synthesized, of which 27 are new compounds. The molecular structures and stereochemistry of the newly synthesized quinine derivatives were confirmed by IR, ^{1}H and ^{13}C NMR spectroscopy (HSQC, HMBC, COSY, NOESY), mass spectrometry, and X-ray crystallographic analysis.

4. For the first time, quantum-chemical modeling and molecular docking studies were carried out for the newly synthesized quinine alkaloid derivatives. The biological activities of the new derivatives, including antimicrobial, antibacterial, antioxidant, anti-inflammatory, analgesic, and cytotoxic activities, were established. Utility model patents of the Republic of Kazakhstan (Nos. 9011, 10114, 9770, and 8794) were granted for the most biologically active compounds.

Significance of the research results

As a result of the conducted chemical transformations, 27 previously undescribed new derivatives of the quinoline alkaloid quinine were synthesized, and their structures

were established using modern methods of IR, ^{1}H and ^{13}C NMR spectroscopy (HSQC, HMBC, COSY, NOESY), mass spectrometry, and X-ray crystallographic analysis.

The results of quantum-chemical modeling and molecular docking of the synthesized compounds made it possible to elucidate the reactivity and stereochemical features of the polyfunctional quinine molecule, as well as to establish the influence of substituent nature on reaction pathways and their selectivity. The synthetic transformations performed significantly expanded the possibilities for targeted modification of the quinoline alkaloid quinine and deepened the theoretical understanding of its chemical properties and reactivity.

For the first time, the biological activities of the synthesized compounds, including antimicrobial, antibacterial, antioxidant, anti-inflammatory, analgesic, and cytotoxic activities, were investigated, leading to the identification of highly active compounds. Structure–biological activity relationships were established. The most significant new compounds exhibiting high biological activity were protected by patents of the Republic of Kazakhstan. The obtained patents confirm the practical significance of the research results and demonstrate the high potential of further application of these compounds as pharmaceutical substances.

Relationship of the research with scientific projects and state research programs. The dissertation research was carried out at the Faculty of Chemistry of the Non-Profit Joint-Stock Company "Karaganda Buketov University" within the framework of the grant project of the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan No. AP19674667 "Synthesis and chemical transformations of a new type of polyheterocyclic compounds based on quinoline and isoquinoline alkaloids for targeted delivery to biological systems" for the period 2023–2025 (State registration No. 0123RK00214).

The author's personal contribution to the dissertation research consists of an independent analysis of scientific, technical, patent, and specialized literature related to the topic of the study. The author planned and carried out a comprehensive set of chemical experiments, including synthetic transformations, chromatographic separation of reaction mixtures, isolation, and purification of new individual compounds. Structural identification of the synthesized substances was personally performed by the author based on the interpretation of spectral and analytical data. The experimental results obtained were analyzed and discussed in collaboration with the scientific supervisors.

The doctoral student's contribution to the preparation of each publication included conducting literature and patent searches, selecting appropriate scientific journals, preparing and writing the manuscripts, interpreting the obtained results, and corresponding with journal editors and reviewers for the following scientific articles:

1. "Synthesis, Antibacterial Properties and Molecular Docking Studies of Nitrogen Substituted 9-(((4X-But-2-ynyloxy)methyl)-1,2,3-triazolyl)–Cinchona

Alkaloid Conjugates" https://doi.org/10.3390/molecules30224352 – Synthesis of new 9-triazolyl- and amino-substituted cinchona alkaloid derivatives; structure elucidation using IR, ^{1}H and ^{13}C NMR spectroscopy, and mass spectrometry; investigation of antibacterial activity and molecular docking at the FAD-binding site of the MurB enzyme.

2. "Synthesis and Biological Activity of Novel Polyazaheterocyclic Derivatives of Quinine" https://doi.org/10.3390/molecules27217387 – Synthesis of new polyazaheterocyclic quinine derivatives, structural elucidation using spectral and analytical methods, and investigation of their biological activity and structure–activity relationships.

3. "Development and Computational Analysis of New Alkaloid Derivatives as Potential Inhibitors of the SARS-CoV-2 M^{pro}" https://doi.org/10.1016/j.jchromb.2025.124718 – Development of new natural alkaloid derivatives, molecular docking, and quantum-chemical analysis to evaluate their potential as inhibitors of the SARS-CoV-2 main protease (M^{pro}).

4. "Study of the Anti-Inflammatory Activity of a Series of Nitrogen-Containing Heterocyclic Compounds and Comparison of the Obtained Data with the Results of Ab Initio DFT Quantum-Chemical Calculations" https://doi.org/10.29235/1561-8323-2024-68-6-454-459 – Investigation of the anti-inflammatory activity of nitrogen-containing heterocyclic compounds and comparison of experimental data with the results of DFT-based quantum-chemical calculations.

5. "Synthesis and Investigation of Derivatives of the Quinine Alkaloid as Potential Inhibitors of the SARS-CoV-2 Main Protease (M^{pro})" https://doi.org/10.51580/2023-1.2710-1185.01 – Synthesis of new quinine alkaloid derivatives, structural characterization using physicochemical methods, and evaluation of inhibitory activity against the SARS-CoV-2 main protease using molecular docking.

6. "Preparation of New Derivatives of the Quinine Alkaloid and Study of Their Anti-Inflammatory Activity" https://doi.org/10.32014/2025.2518-1491.289 – Synthesis of new quinine derivatives and investigation of their anti-inflammatory activity *in vivo*, as well as analysis of the influence of chemical modification of the alkaloid on the biological effect.

7. Design, DFT, molecular docking and in silico analysis of alkaloid acyl derivatives as potential inhibitors of the SARS-CoV-2 main protease (M^{pro}) https://doi.org/10.29235/1561-8331-2026-62-1-45-54. – Molecular docking and quantum-chemical analysis were performed to evaluate their potential as inhibitors of the SARS-CoV-2 main protease (M^{pro}).

8. Analgesic Activity of a Series of Nitrogen-Containing Heterocyclic Compounds: Experimental Study and Comparison with Ab Initio DFT Quantum-Chemical Calculations https://doi.org/10.52540/2074-9457.2024.2.67 – Investigation of the analgesic activity of a series of nitrogen-containing heterocyclic compounds and

comparison of experimental pharmacological data with the results of ab initio DFT quantum-chemical calculations. (Article in a journal indexed in the RSCI database.)

The main statements, conclusions, and scientific results of the dissertation were presented and discussed at international scientific conferences:

1. (2S,4S,5R)-2- ((R)-(6-Methoxy-1- methylquinolinium-4-yl) ((1-methylpyridinium -4-carbonyl)oxy)methyl)-1-methyl-5-vinylquinuclidinium triiodide with antimicrobial activity Kirov, Russia, 2022, p. 199 – Synthesis of a quaternary ionic derivative of the quinine alkaloid, structure elucidation, and investigation of its antimicrobial activity.

2. Alkaloids as Trailers for the Delivery of Pharmacophoric Structures to Target Cells Kirov, Russia, 2022, p. 56 – Analysis of the potential of natural alkaloids as molecular carriers (trailers) for the targeted delivery of pharmacophoric fragments to target cells.

3. Synthesis and Biological Activity of New Combined Quinine Derivatives Minsk, 2023, pp. 105–108 – Synthesis of new combined derivatives of the quinine alkaloid and investigation of their biological activity with establishment of structure–activity relationships.

4. Synthesis of New 1,2,3-Triazole Derivatives of Quinine Syktyvkar, 2024, p. 7 – Development of synthetic approaches to 1,2,3-triazole derivatives of quinine and confirmation of their structures by spectroscopic methods.

5. Alkaloid Derivatives as Phytopharmaceuticals Syktyvkar, 2024, p. 61 – Consideration of natural alkaloid derivatives as promising components of phytopharmaceuticals with biological activity.

6. Synthesis and Anti-Inflammatory Activity of (1R)-(6-Methoxyquinolin-4-yl)((2S,4S,5R)-5-vinylquinuclidin-2-yl)methyl 2-phenylquinoline-4-carboxylate Syktyvkar, 2024, p. 134 – Synthesis of a new ester based on the quinine alkaloid and investigation of its anti-inflammatory activity in experimental models.

7. A Method for Preliminary Evaluation of the Biological Activity of Organic Compounds by Ab Initio DFT Quantum-Chemical Modeling of Their Structure and Properties Vitebsk, 2025, pp. 275–276 – Development of a method for preliminary prediction of the biological activity of organic compounds based on quantum-chemical modeling of their structure and electronic properties using the DFT method.

8. Synthesis and Antimicrobial Activity of (1R)-(6-Methoxyquinolin-4-yl)((2S,4S,5R)-5-vinylquinuclidin-2-yl)methyl 4,5-dichloroisothiazole-3-carboxylate Karaganda, 2023, pp. 326–328 – Synthesis of a new isothiazole derivative of the quinine alkaloid and investigation of its antimicrobial activity.

9. Analgesic Activity of (R)-(6-Methoxyquinolin-4-yl)((1S,2R,4S,5R)-5-vinylquinuclidin-2-yl)methyl 2-phenylquinoline-4-carboxylate Nizhny Novgorod, 2025, p. 188 – Investigation of the analgesic activity of a new quinine derivative and comparison of the obtained results with the effects of reference drugs.

10. Analgesic Activity of Novel Quinine Derivatives Karaganda, 2025, pp. 133–134 – Study of the analgesic activity of new quinine derivatives and analysis of the influence of chemical modification of the alkaloid scaffold on the pharmacological effect.

Based on the materials of the dissertation research, the following utility model patents of the Republic of Kazakhstan were obtained:

1. Patent of the Republic of Kazakhstan No. 9011 dated 06.02.2024, application No. 2024/0168.2, for the utility model *(1S,2R,4S,5R)-2-(R)- (6-methoxy-1-methylquinolinium-4-yl)(5-phenylisoxazole-3-carbonyl)oxymethyl -1-methyl-5-vinylquinuclidinium diiodide*, exhibiting analgesic activity.

2. Patent of the Republic of Kazakhstan No. 10416 dated 09.02.2025, application No. 2025/0187.2, for the utility model *(2S,4S,5R)-2-(R)-(2-chloroacetyl)(6-methoxy-1-methylquinolinium-4-yl) methyl-1-methyl-5-vinylquinuclidinium diiodide*, exhibiting antimicrobial activity.

3. Patent of the Republic of Kazakhstan No. 9770 dated 21.09.2024, application No. 2024/1155.2, for the utility model *(1S,2R,4S,5R)-2-((R)-((1-benzyl-1H-1,2,3-triazol-4-yl)methoxy)(6-methoxyquinolin-4-yl)methyl)-5-vinylquinuclidine*, exhibiting antimicrobial activity.

4. Patent of the Republic of Kazakhstan No. 8774 dated 03.11.2023, application No. 2023/1099.2, for the utility model *(1R)-(6-methoxyquinolin-4-yl)((2S,4S,5R)-5-vinylquinuclidin-2-yl)methyl 2-phenylquinoline-4-carboxylate*, exhibiting anti-inflammatory activity.

Published monograph:

1. “Synthesis and Analysis of Esters of Heterocyclic Compounds.” Karagandy: LLP Typography Colorprint, 2022. 127 p. ISBN 978-601-08-5483-3