ANNOTATION

Dissertation for the degree of Doctor of Philosophy (PhD) in the specialty 6D060600 - Chemistry

Amanzhan Assel

Synthesis of new biologically active substances based on the alkaloid harmine

General characteristics of the work. The dissertation is devoted to the synthesis of new compounds based on the alkaloid harmine, the development of optimal synthesis methods, and the study of the structure of new harmine derivatives, and the production of new biologically active medicinal compounds on this basis.

Relevance of the work. β-Carboline alkaloids exhibit a broad spectrum of biological activities, including anticancer, antidepressant, neurotropic, analgesic, antibacterial, hepatoprotective, and insecticidal effects, positioning them as promising source for the development of novel drug candidates.

β-carboline alkaloid harmine that is present in the underground part of *Peganum harmala* L., is an accessible and promising starting compound for targeted chemical modification.

Purpose of the study: Development of a rational method for the isolation of alkaloid harmine from *Peganum harmala* L., development of effective methods for the introduction of alkynyl, aromatic, and heterocyclic substituents at carbon atom C-6 and C-8, and establishment of the relationship between the structure of synthesized compounds and their biological activity.

Objectives of the study:

1. Development of an effective method for the isolation of alkaloid harmine from *Peganum harmala* L.;
2. The synthesis of 8-acetylharmine, study of conditions of its condensation with aromatic aldehydes, and cyclization of the formed chalcones with hydrazine hydrate;
3. Study of the stereoselectivity of the reaction between 8-acetylharmine and hydrazine hydrate. Preparation of (Z)-hydrazone of 8-acetylharmine and study of the conditions of its condensation with arylaldehydes;
4. Bromination of 8-acetylharmine; study of the conditions of the Suzuki-Miyaura reaction of 6-bromo-8-acetylharmine with arylboronic acids;
5. Study of the conditions of iodination of harmine and 8-acetylharmine;
6. Cross-coupling of 8-iodoharmine and 6-iodo-8-acetylharmine with TMS-acetylene in toluene (Sonogashira reaction);
7. [3+2] cycloaddition between (alkyl)azide and terminal alkyne introduced into the harmine structure forming 1,2,3-triazole (CuAAC);
8. Molecular docking and bioscreening of new synthesized alkaloid samples;

9. Structure-activity relationship (SAR) analysis of synthesized harmine derivatives. Identification of the most promising alkaloid compounds with potential as lead compounds for drug development.

Methods of the study: Modern methods of organic synthesis were employed in the course of this work, including 1,3-dipolar cycloaddition reactions and cross-coupling techniques. Extraction, precipitation, chromatography, and crystallization were used for the isolation and purification of β-carboline alkaloids. To elucidate the molecular structures of the synthesized compounds, a range of physicochemical methods was applied: ultraviolet (UV) and infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), high-performance liquid chromatography (HPLC), X-ray crystallographic analysis (XRD), elemental analysis, as well as measurements of optical rotation and melting point. Additionally, quantum chemical calculations were conducted to evaluate the reactivity of the compounds. Structure–activity relationships (SAR) within the series of synthesized harmine derivatives were investigated using molecular docking and bioscreening of the newly synthesized compounds.

The main provisions submitted for defense:

1. The percolation method provides comparatively high efficiency of extraction of total alkaloids and the major component—harmine (extraction degree up to 97% of the content in air-dried raw material) from the roots of *Peganum harmala* L.; the optimal extraction conditions are a raw-material particle size of 2–3 mm, double extraction with a solvent-to-solid ratio of 1:10 at 65°C for three hours.

2. Selective introduction of substituents at the C-8 position of harmine via the Friedel–Crafts method allows synthesis of the new derivative 8-acetylharmine in 61% yield. Formylation of harmine with dichloromethyl methyl ether in chloroform at 0°C in the presence of tin(IV) chloride affords 8-formylharmine in 64% yield.

3. By effectively using 5 mL of 25% aqueous sodium hydroxide solution in the Claisen–Schmidt condensation of 8-acetylharmine with aromatic aldehydes in ethanol at room temperature, the yields of the corresponding chalcones reach 90–95%. Treatment of β-cabolin chalcones with hydrazine hydrate and acetic acid allows the synthesis of harmine derivatives containing a 5-aryl-1-acetylpyrazoline substituent at position C-8 in yields of 56–78%.

4. Reaction of hydrazine hydrate with 8-acetylharmine in an alcoholic medium at a reaction-mixture temperature of 60°C produces Z-hydrazone-8-acetylharmine, and its condensation with aromatic aldehydes affords 2,3-diazines in the (Z,Z) configuration in 56–82% yields.

5. Selective formation of 6-bromo-8-acetylharmine in 90% yield proceeds smoothly upon treatment of 8-acetylharmine with N-bromosuccinimide in a two-equivalent molar ratio in methylene chloride.

6. Synthesis of 6-aryl-8-acetylharmine via cross-coupling of 6-bromo-8-acetylharmine with arylboronic acids occurs upon reflux in aqueous toluene (1:5) in the presence of $Pd(PPh_3)_4$ catalyst, Na_2CO_3 base, and $Bu_4N^+Br^-$ as an additive.

The main results of the research include: the development of an efficient method for the isolation of harmine from *Peganum harmala* L. by percolation; the establishment of effective synthetic routes to 8-formyl- and 8-acetylharmine derivatives of β-carboline alkaloids; the synthesis of chalcones via condensation of 8-acetylharmine with aromatic aldehydes, followed by conversion to 3-substituted 1-acetylpyrazolines upon reaction with hydrazine hydrate in acetic acid; a harmine-based method for synthesizing (Z)-hydrazone-8-acetylharmine and the exploration of its reactivity toward aldehydes; the development of selective halogenation conditions for palladium-catalyzed cross-coupling reactions; and the investigation of conditions for click chemistry based on copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC).

In total, 35 novel alkaloid compounds were synthesized. Molecular docking and bioscreening of the synthesized samples identified promising candidates for the development of new pharmaceutical substances.

Scientific novelty of the work:

1. Effective methodologies for the selective introduction of substituents at the C-8 position of harmine have been developed.

- It has been shown that formylation of harmine with dichloromethyl methyl ether in chloroform in the presence of tin(IV) chloride leads to formation of 8-formylharmine.

- Upon acetylation of harmine with acetyl chloride in methylene chloride at reduced temperature in the presence of tin(IV) chloride, 8-acetylharmine is smoothly obtained.

- Conditions for the hydrazinolysis of 8-acetylharmine have been proposed, proceeding with formation of (Z)-hydrazone-8-acetylharmine, the condensation of which with aromatic aldehydes affords 8-[1-(arylidenehydrazono)ethyl]-substituted harmine derivatives in the (Z,Z) configuration.

- Efficient approaches to the synthesis of harmine derivatives bearing a (5-aryl-1-acetyl-4,5-dihydro-pyrazol-3-yl) substituent at C-8 have been developed via sequential condensation of 8-acetylharmine with aromatic aldehydes and reaction of the resulting β-carboline chalcones with hydrazine hydrate.

2. Conditions for the selective bromination of 8-acetylharmine, yielding 6-bromo-8-acetylharmine, have been proposed. It has been demonstrated that the cross-coupling reaction of 6-bromo-8-acetylharmine with arylboronic acids proceeds readily upon reflux in aqueous toluene (1:5) in the presence of $Pd(PPh_3)_4$ as catalyst, Na_2CO_3 as base, and the ammonium salt $Bu_4N^+Br^-$ (1 equiv); addition of the ammonium salt initiates the cross-coupling.

3. Preparative syntheses of 6-iodo-8-acetylharmine and 8-iodoharmine based on iodination with N-iodosuccinimide in a trifluoroacetic acid–methylene chloride mixture (1:4) have been proposed. A method for the synthesis of 6-ethynyl-8-acetylharmine via Sonogashira coupling of 6-iodo-8-acetylharmine with trimethylsilyl acetylene in toluene, followed by desilylation, has been developed. Selective modification of 6-ethynyl-8-acetylharmine through introduction of a 1,2,3-triazole heterocyclic substituent at C-6 of the β-carboline scaffold has been

accomplished via CuAAC reaction of 6-ethynyl-8-acetylharmine with azides of various origin.

The structures of the newly synthesized molecules were established by IR, UV, ^{1}H, ^{13}C (HSQC, HMBC, COSY, NOESY) NMR and mass spectrometry, elemental and X-ray structural analyses.

By bioscreening, the new C-6 and C-8 substituted harmine derivatives were found to be 4–7 times less toxic at 100 μg/mL toward Artemia salina Leach.

- In neurotropic activity studies using the elevated plus-maze and open-field tests, 8-acetylharmine, harmine azomethines, the β-carboline-chalcone hybrid and 6-(1-aryl-1,2,3-triazol-4-yl) derivatives of 8-acetylharmine at 10 mg/kg exhibited anxiolytic effects.

- In antidepressant activity assays (Porsolt test), harmine displayed a pronounced antidepressant effect, surpassing that of the reference drug amitriptyline at 10 mg/kg; results for 4-fluorohydrazoneharmine and 6-aryl-substituted derivatives of 8-acetylharmine were comparable to those of the reference amitriptyline administered at the same dose.

- In analgesic activity tests (“acetic acid writhing” assay), (Z)-hydrazone-8-acetylharmine and 6-(1-aryl-1,2,3-triazol-4-yl) derivatives of 8-acetylharmine demonstrated effects comparable to those of diclofenac sodium.

The most promising compounds, based on their combined properties, are 8-acetylharmine, (Z)-hydrazone-8-acetylharmine, the β-carboline-chalcone hybrid and 6-(1-aryl-1,2,3-triazol-4-yl) derivatives of 8-acetylharmine, which may serve as prototypes for the development of new multitargeted pharmaceuticals.

The significance of the research findings lies in the identification of patterns and specific features of the synthetic transformations of the alkaloid harmine leading to the formation of structurally diverse 8-substituted derivatives. The study introduces novel approaches to chemical modification at the C-6 and C-8 positions of the harmine scaffold. The application of palladium-based catalytic systems for cross-coupling reactions has provided new insights into the reactivity of polyfunctional molecules. The transformations performed in this work open new avenues for the functionalization of the aromatic ring of harmine and contribute to a deeper theoretical understanding of the chemical properties of this readily available β-carboline alkaloid.

Preliminary biological evaluation of the synthesized harmine derivatives for cytotoxic, analgesic, neurotropic, and antidepressant activities revealed promising compounds with potential as analgesic and antidepressant agents for further investigation.

Connection of the work with the plan of state scientific programs.

The dissertation was carried out at JSC ‘RPC ‘Phytochemistry’ on the topics: №AR05135304 “Chemical study of alkaloid-bearing plants as promising sources of biologically active substances” for 2018-2020 and №AR08052389 “Development of a new neurotropic drug: pharmacological and clinical studies” for 2020-2022.

The author's personal contribution to the dissertation includes the analysis of scientific, technical, and patent literature. The author independently planned and

carried out chemical experiments, chromatographic separation of reaction mixtures, isolation of novel individual compounds, and structural elucidation of synthesized substances using spectroscopic data. The obtained results were discussed with academic advisors. Based on the materials of the dissertation, the author prepared scientific articles and conference abstracts.

The author's contribution to the preparation of each publication involved literature and patent searches, selection of appropriate scientific journals, preparation and composition of manuscripts, interpretation of experimental results, and correspondence with journal editors and reviewers. The contributions are reflected in the following scientific articles:

1. «Synthetic modifications of carboline alkaloid harmine: Synthesis of 8-substituted derivatives» https://doi.org/:10.1007/s10593-019-02429-1 - Synthesis of harmine derivatives at the C-8 position and structure elucidation based on physicochemical data.

2. «Structure and stereochemistry of a hydrazone derivative of harmine» https://doi.org/:10.1134/S0022476621030161 – Synthesis of Z-hydrazone-8-acetylharmine and structural characterization via X-ray crystallographic analysis following ethanol crystallization.

3. «Analgesic and antidepressant activity of 8-substituted harmine derivatives» https://doi.org/:10.1007/s10593-022-03092-9 - Synthesis of novel harmine derivatives and structure elucidation using IR, UV, ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, elemental analysis, optical rotation, and melting point determination.

4. "Synthesis of 6-aryl-substituted derivatives of 8-acetylharmine and evaluation of their cytotoxicity and antidepressant activity» https://doi.org/:10.1007/s10600-024-04530-0 - Synthesis of 6-aryl-substituted 8-acetylharmine derivatives via Suzuki–Miyaura cross-coupling of monohalogenated harmine with phenylboronic acid derivatives. Assignment of ^{1}H and ^{13}C NMR signals to determine molecular structures.

5. «Synthesis and structure of hydrazone derivatives of harmine» https://doi.org/10.32014/2020.2518-1491.48 - Synthesis and NMR (^{1}H and ^{13}C) signal assignments for structure determination of new harmine derivatives.

6. «Neurotropic activity of plant alkaloids» https://doi.org/10.26577/eb.2021.v88.i3.14 - Preparation of harmine for the study of its neurotropic activity.

7. «Synthesis of new acetylharmine derivatives and their neurotropic activity» https://doi.org/10.26577/IJBCh20251811 - // Synthesis and NMR (^{1}H and ^{13}C) signal assignments for structure determination of new harmine derivatives, study of the neurotropic activity of derivatives.

The main findings and scientific results of the dissertation were presented and discussed at the following international conferences:

1. Моделирование способа выделения гармина из сырья *Peganum harmala* L. – Barnaul, 2021. Optimization of harmine isolation procedure (article in a peer-reviewed journal in the RSCI database).

2. Синтез новых соединений на основе гармина и их цитотоксичность. – Syktyvkar, 2024. Synthesis of novel harmine derivatives for cytotoxicity evaluation.

3. Синтез ацетиленовых производных гармина и триазолов на их основе. – Novosibirsk, 2023. Cross-coupling of iodinated derivatives with trimethylsilylacetylene followed by desilylation and subsequent CuAAC reaction with azides to form triazoles.

4. Синтез и структура нового 8-арилакрилоилпроизводного гармина. – Karaganda, 2023. Synthesis and NMR (^{1}H, ^{13}C) signal assignments for structure determination.

5. Новые биологически активные производные 8-ацетилгармина. – Sheregesh, Kemerovo Region, 2022. Synthesis of new derivatives and determination of neurotropic activity.

6. Новые гетероциклические соединения на основе гармина. Строение и биологическая активность. – Perm, 2022. Synthesis and antimicrobial activity evaluation.

7. 6,8-Дизамещенные производные гармина, обладающие нейротропной активностью. – Novosibirsk, 2022. Synthesis and structural elucidation of new 6,8-disubstituted harmine derivatives.

8. New hydrazone derivatives of harmine – Shanghai, 2019. Synthesis of hydrazone-based harmine derivatives.

Publications and approbation of the work. The main provisions of the dissertation are reflected in the following publications: 3 articles in journals recommended by the Science and Higher Education Quality Assurance Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan; 4 articles in international scientific journals indexed in the Web of Science and Scopus databases; 1 article in a journal included in the Russian Science Citation Index (RSCI); Abstracts of 7 reports published in the materials of international conferences.

The main provisions, conclusions, and scientific results of the dissertation were presented and discussed at international conferences: the XIII International Scientific Conference "Chemistry and Technology of Plant Substances" (Syktyvkar, 2024); the All-Russian Scientific Conference with International Participation "Modern Problems of Organic Chemistry" (Novosibirsk, 2022 and 2023); the VII International Scientific and Practical Conference "Theoretical and Experimental Chemistry" (Karaganda, 2023); the All-Russian Youth Scientific School-Conference "Current Issues of Organic Chemistry" (Sheregesh, Kemerovo Region, 2022); the VII All-Russian Conference with International Participation "Technical Chemistry: From Theory to Practice" (Perm, 2022); and the XIII International Conference "Current Issues of Chemistry, Biology and Technology of Natural Compounds" (Shanghai, China, 2019).