ANNOTATION

to dissertation work on the topic **«Chemical development of a biologically active substance based on piperidine containing esters and amides of fluorobenzoic acids»** for the degree of Doctor of Philosophy (PhD) on specialty 6D074800 – «Technology of pharmaceutical production»

of Issayeva Ulzhalgas Bakhytzhankyzy

Relevance of the research topic

By Decree of the Government of the Republic of Kazakhstan dated November 24, 2022 №945, a comprehensive plan for the development of the pharmaceutical and medical industry for 2020-2025 is being implemented from 2020. The absence of scientific research centers and pharmaceutical clusters in the country for the development of innovative medicines and medical products, the low level of innovative technologies, high-tech innovative medicines and medical products, the insufficiency of laboratories for preclinical research and laboratories for medical testing, the insufficiency of trained scientific personnel and workers for pharmaceutical industries in accordance with international standards (GMP) hinders the development of the pharmaceutical industry in the country.

Among the large number of types of organic substances with anti-infective and other pharmacological activity, saturated nitrogenous heterocycles, including piperidine derivatives, are in the lead. In addition, they form the structural basis of natural compounds: alkaloids, azasteriodes, neurotoxins, etc. In this regard, the search and creation of new drugs for shock infection, the search for new synthons capable of reacting to carry out targeted synthesis, and the study of the relationship of structure and activity between new piperidine derivatives are becoming relevant.

Saturated azaheterocyclanes, due to their high potential for biological action and technological simplicity of synthesis, are the objects of research by many major global pharmaceutical companies and university research centers. The main achievements of this area of azaheterocyclic chemistry are the synthesis of multifunctional derivatives of substituted azaheterocycles, which attract the interest of chemists around the world due to the wide range of biological activity they exhibit. The introduction of other pharmacophore fragments into the molecule of azaheterocyclane derivatives leads to the appearance of both expected and unexpected types of biological activity in them.

The relevance of the research of the dissertation work is determined by the following main factors: the need to create biologically active drugs with minimal side effects for practical medicine, as well as to deepen and expand theoretical concepts related to solving issues of structural features and biological action among the studied azaheterocyclanes and their modified derivatives using wide possibilities of fine organic synthesis to obtain pharmacologically active compounds.

Purpose of the dissertation research:

Chemical development of a potentially biologically active substance based on C- and N-substituted piperidines, cyclic amines, by introducing fragments into the molecule that give the molecule anti-infectious, including antimicrobial/fungicidal properties, development of a technological scheme for obtaining.

Objectives of the research:

1. Synthesis of piperidine-containing esters and amides of fluorobenzoic acids, production of their water-soluble forms;

2. Investigation of the spatial structure of the obtained compounds using physico-chemical methods of IR, ^{1}H and ^{13}C NMR spectroscopy, thin-layer chromatography (TLC), elemental microanalysis;

3. *In vitro* determination of anti-infective/antimicrobial/fungicidal activity, cytotoxicity, acute toxicity of compounds;

4. Standardization, determination of stability and quality specifications of a potentially biologically active substance.

5. Development of a technological scheme for obtaining a biologically active substance.

Methods of research:

Methods of classical organic chemistry, physico-chemical, biological, pharmaceuticals-technological, pharmacological, statistical.

Objects of research

N-alkyl-, N-alkoxyalkyl-, N-arylalkyl-4-oxopiperidines, 4,4-disubstituted derivatives of piperidines, amides of fluorobenzoic acids with potential pharmacological activity.

On the way to achieving this goal, it is envisaged to solve a number of synthetic and analytical tasks (determining the fine spatial structure of synthesized structures, clarifying the effect of introducing the nature and position of various substituents into the structure of the piperidine cycle and amides on biological activity).

The subject of the study

Chemical processes leading to the production of research objects and target products of their chemical transformations, as well as the study of the fine structure and properties of synthesized compounds.

Synthesis of piperidine-containing esters and amides of fluorobenzoic acids, preparation of their water-soluble salts, determination of their fine structure using a complex of modern physico-chemical methods, study of anti-infective, including antibacterial, antimicrobial, fungicidal, cytotoxic activity of compounds, identification of the relationship "structure-biological activity". Standardization of a potentially biologically active substance and development of its production technology.

The main provisions submitted for protection

1) new theoretical and applied aspects of the development of chemistry of saturated nitrogenous heterocycles, cyclic amines, directed molecular design of new pharmacologically active C- and N-substituted piperidines and fluorinated amides as potential anti-infective drugs, their structural features and conformational analysis;

2) new synthons for the production of potentially biologically active substances;

3) recommendations for in-depth medical and biological research in order to obtain anti-infective drugs based on them:

- *AIP-15, AIP-16, AIP-17, AIP-21, AIP-23* and *AIP-29,* as substances for the control of *Staphylococcus aureus* pathogens;

- *AIP-23,* as substances to combat the pathogen *Escherichia Coli*;

- *AIP-19, AIP-20* and *AIP-21* as substances to control the pathogen *Candida albicans;*

- *AIP-18, AIP-24, AIP-27, AIP-28* as antiviral agents.

4) «structure-activity» relationship;

5) technological scheme for the production of biologically active substance hydrochloride 1-benzyl-4-(*p*-fluoro-benzoyloxyimino)piperidine (AIP-15);

6) The results of studying the stability, toxicity and quality specification of the biologically active substance hydrochloride 1-benzyl-4-(*p*-fluoro-benzoyloxyimino)piperidine (AIP-15).

Scientific novelty of the research

Currently, there are a huge number of piperidine derivatives with unique pharmacological properties, but interest in the chemistry of piperidine has not waned so far. In this work, the molecular design of new potential pharmacologically active γ-piperidones with alkyl-, alkoxyalkyl-, arylalkyl substituents in the nitrogen atom of the piperidine cycle is carried out. The design was carried out in two directions:

1) targeted modification of starting aminoketones, amines, taking into account the database on the pharmacological activity of the leading compounds (prototypes).

2) the construction of structures by introducing new pharmacophore fragments into the starting molecules.

To obtain the target structures, preparatively simple methods of fine organic synthesis were used - reduction with sodium borohydride, ethinylation under the conditions of the Favorsky reaction, oxymation, cyanhydrin synthesis, acylation of secondary and tertiary piperidoles, piperidine ketoximes, piperidine carboxylic acids, acylation of amines with *para-*, *meta-*, *ortho*-fluorobenzoyl chlorides according to Schotten-Bauman.

The end products of the modifications carried out are potential anti-infective, including antimicrobial/fungicidal and antibacterial drugs.

Modifications were carried out with the participation of a carbonyl group located at the C-4 piperidine cycle, hydroxyl groups, as well as an amino group in cyclic amines.

The building blocks introduced into the molecule of the initial γ-piperidones and leading to biologically active compounds are ethinyl, hydroxyl, carboxyl groups, an oxime fragment, acyl residues of various carboxylic acids - adamantanecarbon, *o-*, *m-*, *p*-fluorobenzoic acids.

Conditions for the acylation of synthesized piperidoles, as well as amines, have been developed, which directionally lead to new piperidine-containing derivatives and fluorinated amides.

Based on the data of IR spectroscopy and ^{13}C NMR spectroscopy, the structure of the obtained compounds was determined.

Among the synthesized compounds, a number of substances with high anti-infectious activity were identified. The most active of them are recommended for in-

depth medical and biological study in order to create anti-infectious agents based on them.

During the conducted microbiological studies in in vitro experiments, it was shown that the following compounds can be isolated for further research on clinical strains:

- *AIP-15, AIP-16, AIP-17, AIP-21, AIP-23* and *AIP-29*, as substances for the control of *Staphylococcus aureus* pathogens;
- *AIP-23*, as substances to combat the pathogen *Escherichia Coli*;
- *AIP-17, AIP-19, AIP-20* and *AIP-21* as substances to control the pathogen *Candida albicans;*

As a result of studying the combined effects of antimicrobial comparison drugs and ligands, it was found that *AIP-15* exhibits a synergistic effect in combination with gentamicin against the multi-resistant *Staphylococcus aureus* ATCC BAA-39 test strain.

Compounds AIP-15, AIP-19, AIP-20 and AIP-21 in combination with nystatin also show a synergistic effect against fungi of the genus *Candida*, with these combinations, the values of the minimum suppressive concentrations of the antimycotic decrease by up to 4 times.

Compounds AIP-18, AIP-24, AIP-27 and AIP-29 showed antiviral activity against the A/H1N1 virus at 1/2 of the maximum studied concentration of CTK_{50}.

1-benzyl-*p*-fluorobenzoyloxy ketoxympiperidine hydrochloride (AIP-15) has been standardized, its stability and acute toxicity have been studied.

The practical significance of the work

First of all, it consists in the accumulation and development of scientific ideas about piperidine compounds, methods of their synthesis, structure, and properties, which can later be used in the purposeful design and identification of even more complex systems and will help predict their behavior. The second, but no less important achievement of the conducted research is the prospects for the development of further research on the chemistry of 4,4-disubstituted derivatives of piperidine and amides. The synthesized compounds were tested at the Department of Pharmaceutical Technologies of the Kazakh National Medical University named after S.D. Asfendiyarov, in the laboratories of microbiology and Virology of JSC Scientific Center for Anti-Infectious Drugs, in the Laboratory of Microbiology of JSC MNPH "Phytochemistry", Karaganda. As a result of pharmacological screening, it was found that a number of compounds exhibit anti-infectious activity, and were recommended for in-depth study of their pharmacological properties.

Author's personal contribution

On the topic of the dissertation work, the dissertation conducted an independent review and analysis of domestic and foreign literature, carried out practical work on all the tasks set. This is confirmed by the results of studies obtained in laboratory and production conditions using modern equipment and literature. The reliability and validity of the research results is confirmed by the focus on solving the actual problem of the work performed, the implementation of regulatory documents in a modern research center and projects.

Conclusions

A number of potentially biologically active cyclic amides of fluorobenzoic acids have been synthesized according to Schotten-Bauman.

On the basis of acetylene (cocaine) alcohol, the corresponding esters of para-, meta-, ortho-fluorobenzoic acids were synthesized by acylation of *4-fluoro-, 3-fluoro-, 2-fluoro*benzoyl chlorides.

Chemical modification of molecules of a number of N-substituted 4-oxopiperidines and leader compounds was carried out in order to obtain new innovative pharmaceutical substances (ligands). 4,4-disubstituted derivatives have been synthesized on the basis of N-(alkoxyalkyl-)-4-oxopiperidines. Piperidine-containing esters of adamantanecarboxylic acid have been synthesized.

In order to search for new drugs in a number of piperidine derivatives based on [1-(2-ethoxyethyl)-, 1-(3-ethoxypropyl)-, 1 benzyl]-piperidine-4-ones by reduction of sodium borohydride in isopropanol with good yields, appropriate secondary alcohols have been developed.

On the basis of [1-(2-ethoxyethyl)-, 1-(3-ethoxypropyl)-, 1-benzyl-]-4-hydroxypiperidines, acylation of 3-fluoro-, 2,6-difluoro4-(trifluoromethyl)-benzoyl chlorides was carried out with the formation of corresponding hydrochlorides of esters of fluorobenzoic acids having an aromatic cycle one, two and three fluorine atoms.

Ketoxympiperidine was synthesized and developed as an intermediate intermediate of the reaction for further transformations by the interaction of hydrochloric acid hydroxylamine with 1-benzyl-4-oxopiperidine in the presence of alkali in ethanol.

In order to introduce one, two and three fluorine atoms, naphthyloxygroups and an adamantane fragment into the structures of piperidines, as well as to clarify the effect on the anti-infectious activity of compounds by acylation of the resulting ketoxime 1-benzyl-4-oxopiperidine with 4-fluoro-benzoyl chloride, 3-fluoro-benzoyl chloride, 2-fluoro-benzoyl chloride, 2,6-difluorobenzoyl chloride, 4-(trifluoromethyl) benzoyl chloride synthesized hydrochlorides of esters of fluorobenzoic acids, and also by acylation of N-benzylpiperidine ketoxime with naphthoylchloride and adamantantancarbonyl chloride, the corresponding hydrochlorides of naphthoic and adamantancarboxylic acid esters were obtained.

Cyanhydrin synthesis, as the simplest preparative method of introducing a carboxyl group into a piperidine molecule, is used to obtain potential biologically active piperidine carboxylic acids having alkyl and arylalkyl radicals in the nitrogen atom of the piperidine cycle. Crystalline cyanhydrins of [1-methyl, 1-propyl-, 1-benzyl-, 1-(2-phenylethyl-)]-4-ketopiperidines with yields of 75.5-83.3% were obtained by reacting the corresponding piperidine-4-ones with acetone cyanhydrin. The reaction was carried out at room temperature without solvent, with the addition of a small amount (2-3 drops) of water. Further, in order to obtain amino acids, their acid hydrolysis was carried out to form [1-methyl, 1-propyl-, 1-benzyl-, 1-(2-phenylethyl-)]-4-hydroxy-4-carboxypiperidines. Cyanhydrin hydrolysis was carried out with concentrated hydrochloric acid and took place at room temperature.

In order to study the reactivity of the hydroxyl group and in order to study the reactivity of the hydroxyl group and subsequently clarify the effect of the nature of the acyl residue, in particular the introduction of a fragment of cyclopropane and a fluorophenyl fragment into the structures of piperidines on pharmacological properties, esters of cyclopropancarboxylic and *p*-,*m*-,*o*-fluorobenzoic acids on the hydroxyl group of N-substituted hydrochlorides were synthesized 4-hydroxy-4-carboxypiperidines.

The structure of the synthesized substances was established on the basis of IR spectroscopy and ^{1}H and ^{13}C NMR spectroscopy data.

The introduction of a fluorine atom in piperidine, an ethoxyethyl radical into a nitrogen atom and a triple bond in the fourth position caused antimicrobial activity. The introduction of a fluorine atom into the structure of kazkain led to the manifestation of antimicrobial activity, the fluorine atom in the ortho position turned out to be optimal, therefore *o*-fluorobenzoate 1-(2-ethoxyethyl)-4-ethyl-4-hydroxypiperidine hydrochloride showed antimicrobial activity. activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* yeast demonstrated a relatively wide range.

The introduction of an adamantane fragment into the structure of N-alkoxyalkylpiperidines showed high antimicrobial activity, in particular, 1-(2-ethoxyethyl-)-4-adamantancarbonyloxypiperidine hydrochloride (AIP-5, 2.21) and 1-(3-ethoxypropyl-)-4-adamantancarbonyloxy piperidine hydrochloride (AIP-6, 2.22) 125 mcg/at concentrations in ml and 250 mcg/ml, Staphylococcus aureus showed the greatest antimicrobial (bactericidal) activity against the ATCC 6538-P strain, the compound AIP-5 showed activity 16 times more than the comparison drug ampicillin, and AIP-6- 8 times.

The compound AIP-5 showed the greatest activity against the museum test strain *Escherichia coli* ATCC 8739 at a concentration of 500 micrograms/ml, i.e. 4 times higher than that of ampicillin.

It was found that the fungicidal activity against *Candida albicans* ATCC 10231 culture in heteroorganic derivatives of AIP-5 at a concentration of 250 mcg/ml and AIP-6 at a concentration of 500 mcg/ml is higher than that of the compared drug fluconazole AIP-5 by 10 times and AIP-6 by 5 times.

The antimicrobial (antimicrobial and fungicidal) activity in vitro of heteroorganic derivatives AIP-15, AIP-16, AIP-17, AIP-18, AIP-19, AIP-20, AIP-21, AIP-22, AIP-23, AIP-24, AIP-25, AIP-26 was studied in relation to museum test strains.

Heteroorganic derivatives AIP-15, AIP-16, AIP-17, AIP-23, AIP-29 and AIP-21 showed antimicrobial (bactericidal) activity to the museum test strain *Staphylococcus aureus* ATCC 6538-P in concentrations from 1000 mcg/ml to 125 mcg/ml.

Thus, from the studies conducted to study the antimicrobial action of new heterorganic derivatives, we can note AIP-15+gentamicin, AIP-15+amoxicillin, AIP-16+gentamicin, AIP-19+nystatin, AIP-20+nystatin, AIP-21+nystatin together with comparable substances. These combined test substances lead to a synergistic effect, reducing their MBC several times.

Derivatives A IP-23, IP-29 showed activity against the museum test strain *Escherichia coli* ATCC 8739 at concentrations of 500 mcg/ml and 1000 mcg/ml, respectively.

Fungicidal activity against *Candida albicans* ATCC 10231 culture was established in heteroorganic derivatives AIP-18, AIP-22, AIP-23 at a concentration of 1000 mcg/ml, AIP-15, AIP-16, AIP-17 at a concentration of 500 mcg/ml, AIP-21 and AIP-24 at a concentration of 250 mcg/ml, in AIP-19 and AIP-20 at a concentration of 125 mcg/ml.

The introduction of the p-fluoro-benzoyloxygroup and m-fluorobenzoyloxygroup into the structure of N-benzylpiperidine ketoxime led to highly effective antimicrobial drugs, so of all 15 studied compounds for anti-infective activity in an in vitro experiment, only two compounds, in particular, compound 2.35 under the cipher AIP-15 (N-benzylpiperidine ketoxime para-fluorobenzoic hydrochloride) and compound 2.36 under the cipher AIP-16 (N-benzylpiperidine ketoxime *meta*-fluorobenzoic hydrochloride esters turned out to be highly effective drugs in concentrations from 31 micrograms/ml to 63 micrograms/ml against the multidrug-resistant strain *Staphylococcus aureus* ATCC BAA-39 bactericidal activity was determined accordingly.

The bactericidal activity of AIP-23 at a concentration of 250 micrograms/ml has been established in relation to the multidrug-resistant strain of *Escherichia coli* ATCC BAA-196.

When studying in vitro the combined action of the antifungal drug nystatin with heteroorganic derivatives against the reference strain *Candida albicans* ATCC 10231, it was found that AIP-19, AIP-20 and AIP-21 exhibit a synergistic effect, reducing the values of the minimum fungicidal concentrations of the two components.

Mutual enhancement of antimicrobial activity is also observed with the combined action of AIP-15 with gentamicin, AIP-15 with amoxicillin and AIP-16 with gentamicin against the multiply resistant *Staphylococcus aureus* ATCC test strain BAA-39 manifested in the form of synergy.

Cytotoxicity of *in vitro* heteroorganic derivatives AIP-15, AIP-16, AIP-17, AIP-18, AIP-19, AIP-20, AIP-21, AIP-22, AIP-23, AIP-24, AIP-25, AIP-26, AIP-27, AIP was studied-28 and AIP-29 on the MDCK cell line. It has been shown that of all the studied heteroorganic derivatives, compounds AIP-17, AIP-25, AIP-26, AIP-27 and AIP-28 have the least toxicity to MDCK cells.

The therapeutic activity of in vitro heteroorganic derivatives AIP-15, AIP-16, AIP-17, AIP-18, AIP-19, AIP-20, AIP-21, AIP-22, AIP-23, AIP-24, AIP-25, AIP-26, AIP-27, AIP-28 was studied, AIP-29 for influenza A/Swine/Iowa/30 (H1N1) virus. It was shown that the AIP-28 compound suppressed the replication of 100 infectious doses of the virus on

1.2 log2 compared with the control group only in the maximum studied concentration – 1.85 mg/ml. The AIP-18 compound suppressed replication of 100 infectious doses of the virus by 2.0 log2 compared with the control group at concentrations of 0.065 and 0.0325 mg/ml. The compound AIP-24 at a concentration of 0.035 mg/ml suppresses 100 infectious doses of influenza A/H1N1 virus by 2.0

log2, and at concentrations of 0.0175 and 0.0088 mg/ml by 1.0 log2. The AIP-27 compound is capable of suppressing 100 infectious doses of the virus by 1.0 log2, and the AIP-29 compound by 2.0 log2 only at the maximum used concentrations of 4.75 mg/ml and 0.08 mg/ml, respectively.

Approbation of the results of the dissertation

The main results of the dissertation research are presented at the following conferences: XIV International Scientific and Practical Conference of Young Scientists and Students "Scientific discussion: topical issues, achievements and innovations in medicine" (Dushanbe, Tajikistan, April 19, 2019), XX International Scientific and Practical Conference of Students and Young Scientists "Chemistry and Chemical Technology in the XXI century" (Tomsk, May 2019), International Scientific and practical Conference "XXI Mendeleev Congress on General and Applied Chemistry" (St. Petersburg, September 2019); International Scientific and practical Conference "XXI Mendeleev Congress on General and Applied Chemistry" (St. Petersburg, September 2019), International Conference "Modern problems of chemistry and technology of organic substances and materials" (Almaty, 2019), VIII All-Russian conference dedicated to the 60th anniversary of CJSC Khimprom "Topical issues of chemical industry technologies and Environmental Protection" (Cheboksary, April 16-17, 2020), III International Scientific and Practical Conference "Formation and prospects of development of the scientific school of Pharmacy: succession of generations" (Almaty, October 16, 2020), International Scientific and Practical Conference "Trends, prospects and innovative approaches to the development of chemical science, production and education in the context of globalization" (Almaty, November 3, 2021), International Scientific and Practical Conference "Fine Organic Synthesis-2021" (Almaty, September 3, 2021), International scientific conference of young scientists "Science and Innovation" (Tashkent, October 20, 2022), Forum of Young scientists of the CIS member states "Science without Borders" within the framework of the program of events to celebrate the 300th anniversary of the Russian Academy of Sciences (Nizhny Novgorod, 1-November 3, 2022).

Publications

The results of the dissertation research have been published in 24 scientific papers, including:

- 4 articles have been published in publications included in the list approved by the Committee for Control in the Field of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan;
- 1 article published in an international scientific publication included in the Scopus database;
- 17 abstracts and articles published at national and international scientific conferences;
- 2 patents of the Republic of Kazakhstan for a utility model have been obtained.

Connection of work with the plan of state and scientific programs:

The dissertation work was carried out in accordance with the plan of scientific research JSC "Institute of Chemical Sciences at the A.B.Bekturov" on the topics:

"Physico-chemical foundations for the creation of inorganic, organic, polymer compounds, systems and materials with the necessary properties" (Scientific and Technical Program №BR05234667) (2018-2020), "Development of original domestic innovative pharmaceutical substances with anti-infective activity (ligands)" (Grant financing project of the Ministry of Education and Science Republic of Kazakhstan №AP05131065) (2018-2020), "Innovative multifunctional materials based on natural raw materials and man-made waste" (Scientific and Technical Program №BR10965255) (2021-2023).

The reliability and validity of the work

The validity and reliability of the results, conclusions and conclusions formulated in the dissertation related to the synthesis of new piperidine-4-ones and their derivatives is confirmed by the data of a complex of methods of physico-chemical analysis (IR, NMR ^{13}C spectroscopy, elemental analysis, thin-layer chromatography). The results concerning the biological activity of compounds obtained by the author for the first time are confirmed by the results of biological tests of compounds (relevant test certificates are available in the appendix) and security documents for inventions. In the dissertation work, the author applies experimental methods tested in fine organic synthesis and establishes the spatial structure of six-membered cyclic systems.

Scope and structure of the dissertation:

The dissertation contains 204 pages, 55 tables and 15 figures, consists of an introduction, literary review, practical part, discussion of the results of practice, technological part, conclusion, appendix and used literature containing 160 titles.