CHEMISTRY AND TECHNOLOGY OF MEDICINAL COMPOUNDS AND BIOLOGICALLY ACTIVE SUBSTANCES
ХИМИЯ И ТЕХНОЛОГИЯ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ И БИОЛОГИЧЕСКИ АКТИВНЫХ СОЕДИНЕНИЙ

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RESEARCH ARTICLE

Synthesis and biological activity of *N*-phosphonacetyl-L-aspartate's structural analogs *N*-(α-dietoxyphosphorylcyclopropylcarbonyl)-amino acids

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Objectives. *With the development and improvement of new delivery systems for substances of various natures, organophosphorus compounds with an antimetabolic mechanism of action have become relevant again. A few examples of them are organophosphorus analogs of carboxylic acids, such as N-phosphonacetyl-L-aspartate (PALA) and N-phosphonacetyl-L-isoasparagine, both of which are bio-rationally developed analogs of the transition state of carbamoylaspartate in the biosynthesis of pyrimidine bases, which is catalyzed by the enzyme aspartate transcarbamoylase (ATCase). Despite their high activity, these compounds have not found widespread use as anticancer agents due to a large number of side-effects and low bioavailability. Given the emerging opportunities for the delivery of phosphate and phosphonate derivatives into target cells, obtaining more effective analogs of PALA seems to be an interesting and promising research objective. The goal of the present study was thus to synthesize and study the biological activities of novel PALA analogs that are derivatives of phosphonacetic acid.*

Methods. *For directed work within the framework of the study, we used the molecular docking method, which allowed us to simulate the binding of N-(a-diethoxyphosphorylcyclopropylcarbonyl)-substituted amino acids to ATCase. The target compounds were synthesized using classical methods of organic synthesis. The obtained compounds' cytotoxicity was probed in relation to cell lines of human breast cancer (MDA-MB-231), skin cancer (A-375), and glioblastoma (U-87 MG).*

Results. *The synthesis of eight novel N-(a-diethoxyphosphorylcyclopropylcarbonyl)-substituted amino acids was carried out. A few of the synthesized derivatives were tested for anticancer activity, but none displayed significant cytotoxicity.*

Conclusions. *N-(α-diethoxyphosphorylcyclopropylcarbonyl)-substituted amino acids are synthetically available analogs of PALA, a compound capable of strong interaction with ATCase. However, the compounds synthesized in this work did not display any pronounced anticancer properties. One of the reasons for the observed low activity may be the presence of ether groups in the phosphonate building block.*

Keywords: *phosphonocarboxylic acids, N-phosphonacetyl-L-aspartate (PALA), aspartate transcarbamylase (ATCase), α-diethoxyphosphonacetic acid, α-diethoxyphosphorylcyclopropylcarboxylic acid, N-(α-diethoxyphosphorylcyclopropylcarbonyl)amino acids.*

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НАУЧНАЯ СТАТЬЯ

Синтез и биологическая активность *N*-(α-диэтоксифосфорилциклопропилкарбонил)аминокислот – структурных аналогов *N*-фосфонацетил-L-аспартата

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Цели. *С развитием и совершенствованием новых систем доставки для веществ различного характера, вновь приобретают актуальность фосфорорганические соединения с антиметаболитным механизмом действия. К ним можно отнести, например, фосфорорганические аналоги карбоновых кислот, такие как N-фосфонацетил-L-аспартат (PALA) и N-фосфонацетил-L-изоаспарагин, являющиеся биорационально разработанными аналогами переходного состояния карбамоиласпартата в реакции биосинтеза пиримидиновых оснований, которая катализируется ферментом аспартат-транскарбамоилазой (ATCase). Несмотря на высокую активность эти соединения не нашли широкого применения из-за большого количества побочных эффектов и низкой биодоступности. С учетом открывающихся возможностей по доставке фосфатных и фосфонатных производных в клетки-мишени, получение более эффективных аналогов PALA кажется интересной и перспективной задачей. Поэтому целью данной работы являлись синтез и исследование биологической активности новых производных фосфонуксусной кислоты – N-(α-диэтоксифосфорилциклопропилкарбонил)-замещенных аминокислот – аналогов N-фосфоноацетил-L-аспартата (PALA).*

Методы. *Для направленной работы в рамках исследования применяли метод молекулярного докинга, который позволяет смоделировать связывание N-(α-диэтоксифосфорилциклопропилкарбонил)-замещенных аминокислот с аспартат-транскарбамоилазой. Целевые соединения были синтезированы с использованием классических методов органического синтеза. Исследование цитотоксичности проводили по отношению к клеточным линиям рака молочной железы человека (MDA-MB-231), рака кожи (A-375) и глиобластомы (U-87 MG).*

Результаты. *В рамках работы был осуществлен синтез восьми новых N-(α-диэтоксифосфорилциклопропилкарбонил)-замещенных аминокислот. Исследование ряда синтезированных производных на противораковую активность не выявило значимого проявления цитотоксичности.*

Выводы. *N-(α-диэтоксифосфорилциклопропилкарбонил)-замещенные аминокислоты представляют собой синтетически доступные аналоги PALA, способные к более сильному взаимодействию с ATCase. Тем не менее синтезированные в данной работе соединения не проявили выраженных противораковых свойств. Одной из причин низкой активности может быть наличие эфирных групп в фосфонатном структурном элементе.*

Ключевые слова: *фосфонкарбоновые кислоты, N-фосфонацетил-L-аспартат (PALA), аспартат-транскарбомоилаза (ATCase), диэтоксифосфорилуксусная кислота, α-диэтоксифосфорилциклопропанкарбоновая кислота, N-(α-диэтоксифосфорилциклопропилкарбонил)-замещенные аминокислоты.*

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INTRODUCTION

Antineoplastic drugs with an antimetabolic mechanism of activity have become established in the practice of oncological disease treatment. Although these drugs differ in structure, intracellular metabolism, and mechanism of cytotoxic action, all of them, in one way or another, are analogs of natural metabolites, and they are able to compete with these metabolites, both with respect to transport pathways into the cell and participation in key enzymatic processes. Antimetabolites of nucleic acid biosynthesis are especially relevant for the treatment of oncological diseases.

A promising anticancer drug identified in the 1980s was *N*-phosphonacetyl-L-aspartate (PALA) **1**, which was developed as a result of the bio-rational approach (Fig. 1). In fact, the anticancer activity of PALA is based on its structural similarity with the transition state of carbamoyl aspartate, which is involved in the biosynthesis of pyrimidine bases catalyzed by aspartate transcarbamoylase (ATCase) [1–4].

PALA showed high activity against solid tumors and passed two phases of clinical trials [5, 6]. Unfortunately, as is true for many other organophosphorus compounds, the high *in vitro* activity of PALA was not reproduced *in vivo* due to the low bioavailability of this compound and the large number of side-effects associated with its administration [7, 8]. Therefore, the synthesis of new derivatives of phosphonacetic acid with potential anticancer activity and improved pharmacological properties is of high research interest.

Known derivatives of phosphonacetic acid, which are structural analogs of PALA, include compounds **2–4**, whose structures are reported in Fig. 2. These compounds have a lower total charge and improved pharmacological properties than PALA, but they also display significantly lower activity than the mentioned species [9].

Considering the structural similarity of the PALA to the intermediate of carbamoylaspartate biosynthesis, we assumed that the key significance to increase the biological activity of phosphonacetic acid derivatives was the value of the optimal P–C bond angle, which will ensure the stability of the conformation most favorable for the formation of a complex with the active center of the enzyme. Additionally, we

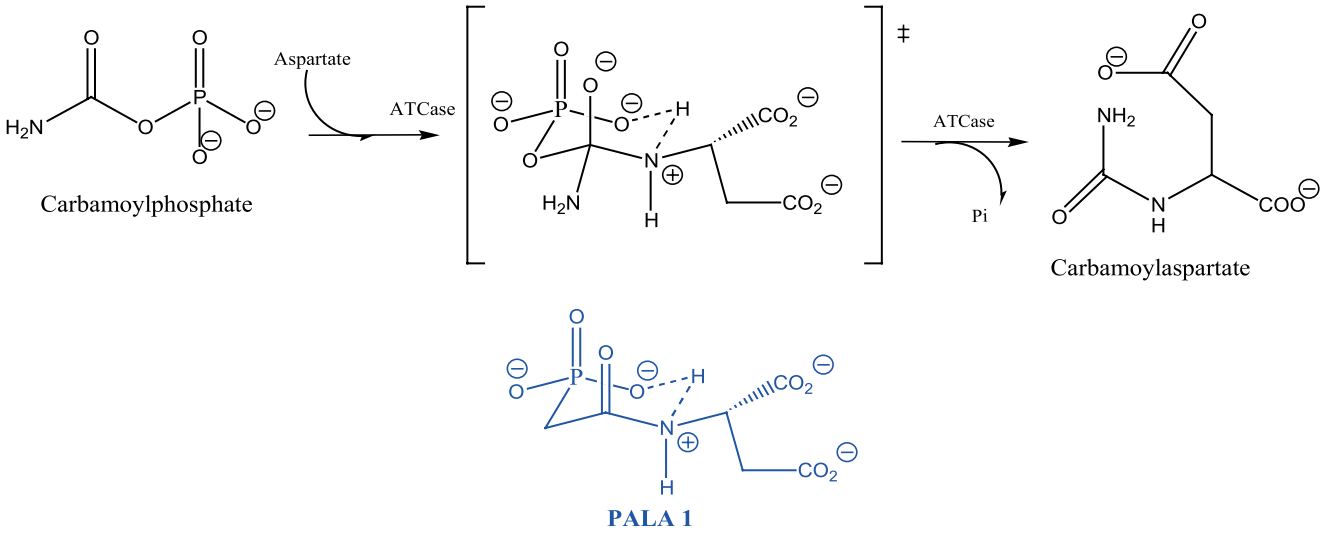


Fig. 1. Structural similarity between *N*-phosphonacetyl-L-aspartate and the intermediate of carbamoylaspartate biosynthesis.

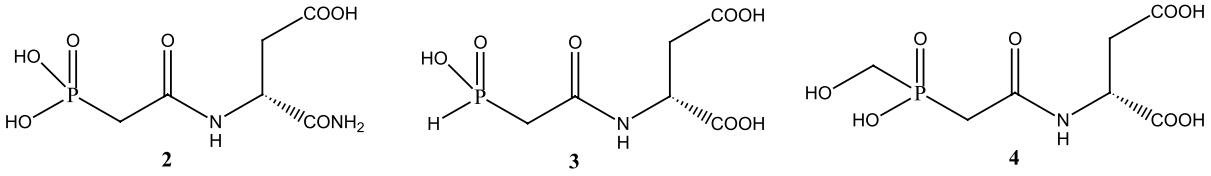


Fig. 2. Structural formulas of *N*-phosphonacetyl-L-aspartate analogs.

considered a pronounced hydrophobic fragment in the structure of a molecule due to the presence of a "hydrophobic pocket" in the ATCase structure, which will increase the strength of the binding between enzyme and the substrate. These objectives may be achieved introducing bulky substituents on the carbon atom of the phosphonoacetate skeleton. Cyclopropanyl-substituted phosphonacetic acids are interesting from the standpoint of synthetic accessibility, variation of the P–C bond angle, and the content of the hydrophobic fragments. In fact, the synthesis and investigation of the anticancer activity of this family of compounds were carried out within the framework of the present study.

MATERIALS AND METHODS

Chromato-mass spectrometric analyses were conducted on a Thermo Fisher Scientific Surveyor MSQ (*Thermo Fisher Scientific*, USA) with a Phenomenex Onyx Monoliythic C18 25 × 4.6 mm high performance liquid chromatography column (*Phenomenex*, USA). A two-component mixture of a 0.1% solution of formic acid and acetonitrile (solvent–100% dimethyl sulfoxide (DMSO), gradient elution, flow rate = 1.5 mL/min, temperature = 25°C, type of ionization used at atmospheric pressure: electrospray) was used as mobile phase. Nuclear magnetic resonance (NMR) ^{1}H spectra were recorded on a Varian MercuryPlus 400 instrument (*Varian*, USA) ($CDCl_3$, DMSO-d_6, tetramethylsilane as internal standard). Melting points were determined on a Stuart SMP20 apparatus (*Stuart*, UK). For thin layer chromatography, we used Merck Thin Layer Chromatography Silica gel 60 F_{254} aluminum plates (size 10 × 20 cm) (*Merck*, Germany). For column chromatography, we used Merck silica gel 60 with a particle size of 0.015 mm to 0.040 mm. A CEM DU 9369 microwave reactor (*CEM Corporation*, USA) was used to carry out reactions under microwave irradiation.

DMSO was distilled over calcium hydride before use; dibromoethane was distilled under reduced pressure; the amino acids used were not pre-purified; triethylamine was distilled over KOH; triethyl phosphite was not pre-purified; extraction solvents were used without any prior treatment.

EXPERIMENTAL

Preparation of triethyl ester of phosphonoacetic acid (5).

In a septum-equipped 10-mL test tube suitable for use in a microwave reactor were placed 3.32 g (0.02 mol) of triethyl phosphite and 2.45 g (0.02 mol) of the ethyl ester of α-chloroacetic acid. The reaction was carried out under 250-W-power microwave irradiation and a temperature of 170°C for 1 h. The isolation of the final product was conducted by vacuum distillation on an oil pump with a yield of 3.85 g (86%). T_{boil} = 110°C (0.1 mm Hg). ^{1}H NMR spectrum (400 MHz, DMSO-d_6): δ (ppm) 4.07 (dq, J = 15.1, 7.1 Hz, 6H), 3.10 (d, J = 21.4 Hz, 2H), 1.22 (dt, J = 18.4, 7.1 Hz, 9H).

Preparation of α-diethoxyphosphorylcyclopropane carboxylic acid ethyl ester (6).

In a 500-mL flat-bottom flask were mixed together 15 g (0.067 mol) of crushed KOH and 100 mL of freshly distilled DMSO. Under stirring, to the resulting suspension were added dropwise in succession 7.48 g (0.03 mol) of diethoxyphosphonoacetic acid ethyl ester and 25.19 g (0.268 mol) of dibromoethane. Another 170 mL of DMSO were then added to the reaction mixture. The resulting suspension was stirred at room temperature for 72 h. In order to separate the reaction products from DMSO, 200 mL of water were added to the reaction mixture, and an extraction was performed using diethyl ether (three consecutive extractions, with 100 mL of solvent at a time) and then chloroform (two consecutive extractions, with 100 mL of solvent at a time). The resulting organic phases were dried over Na_2SO_4, filtered off, combined, and concentrated on a rotary evaporator. The final product was isolated by vacuum distillation on an oil pump with a yield of 5.60 g (67%) . T_{boil} = 110–115°C (0.1 mm Hg). ^{1}H NMR spectrum (400 MHz, DMSO-d_6): δ (ppm) 4.16–3.96 (m, 6H), 1.38–1.27 (m, 1H), 1.21 (dt, J = 16.0, 7.1 Hz, 1H).

Preparation of α-diethoxyphosphorylcyclopropane carboxylic acid (7).

In a 500-mL flat-bottom flask, 20.573 g (0.08 mol) of diethoxyphosphorylcyclopropanecarboxylic acid ethyl ester and 140 mL of 1N (0.14 mol) aqueous KOH solution were added. The mixture thus obtained was stirred for 30 min at room temperature, and it was then acidified with a 20% H_2SO_4 to reach a pH of about 2

(controlled by litmus paper). The mixture was then stirred at room temperature for an additional 30 min. In order to isolate the target compound, an extraction with chloroform (four consecutive extractions, with 50 mL of solvent at a time) was performed. The resulting organic phases were dried over Na_2SO_4, filtered off, combined, and concentrated on a rotary evaporator. The product was purified by recrystallization from diethyl ether with the addition of hexane. White hygroscopic crystals with a yellowish tint weighing 10.7 g were thus obtained, for a reaction yield of 60%. The product's melting point was 85–87°C. ^{1}H NMR spectrum (300 MHz, DMSO): δ (ppm) 4.16 (dq, *J* = 14.2, 7.1 Hz, 4H), 1.45–1.38 (m, 4H), 1.35 (t, *J* = 7.0 Hz, 6H).

Preparation of α-diethoxyphosphorylcyclopropanecarboxylic acid chloride.

In a 25-mL flask were placed 5 g (0.023 mol) of α-diethoxyphosphorylcyclopropanecarboxylic acid and 7 mL (0.23 mol) of $SOCl_2$. The mixture thus obtained was heated under stirring at 50°C for 1 h until gas evolution from the reaction mixture ceased; subsequently, excess $SOCl_2$ was distilled off in a water-jet pump vacuum. The resulting acid chloride was immediately added to the acylation reaction without preliminary purification.

Procedure for the synthesis of α-diethoxyphosphoryl-cyclopropanecarboxylic acid amides 8–11.

In a 100-mL three-neck flask equipped with a thermometer, a dropping funnel, and a reflux condenser comprising a calcium chloride tube, were placed 0.014 mol of dry amino acid ethyl or methyl ester hydrochloride, after it had been ground it in a porcelain dish. About 30 mL of chloroform was then added to the reaction flask, and 0.04 mol of triethylamine were added under stirring using a pipette. The reaction mixture was cooled to 0°C in a bath with ice and salt and stirred for 0.5 h. Afterwards, 0.014 mol of α-diethoxyphosphorylcyclopropanecarboxylic acid chloride were added using a dropping funnel, while not allowing the temperature of the mixture to rise above 5°C. After adding the entire amount of acid chloride, the mixture was stirred for another 0.5 h at room temperature. The solvent of the reaction mixture was then evaporated on a rotary evaporator, and to the residue were added 100 mL of ethyl acetate; the mixture thus obtained was stirred for 10 min at room temperature, and the triethylamine hydrochloride was filtered off. The solvent of the resulting filtrate was again evaporated on a rotary evaporator. A viscous yellow-orange liquid was obtained. The product was purified by column chromatography on silica gel using ethyl acetate as eluent. A viscous liquid characterized by a bright yellow color was obtained.

***N*-(α-diethoxyphosphorylcyclopropylcarbonyl)-glycine ethyl ester (8)**, 3.18 g (74%) yield.

^{1}H NMR spectrum (400 MHz, DMSO-d_6): δ (ppm) 8.04 (t, *J* = 5.5 Hz, 1H), 4.20–4.04 (m, 6H), 3.94 (d, *J* = 5.7 Hz, 2H), 1.29 (t, *J* = 7.1 Hz, 9H), 1.26–1.13 (m, 4H).

Liquid chromatography–mass spectrometry (LC–MS) data, m/z (*I*, %): exp. 308.062 $[MH]^+$, 100%; calc. 308.29 $[MH]^+$.

***N*-(α-diethoxyphosphorylcyclopropylcarbonyl)-aspartic acid dimethyl ester (11),** 3.88 g (76%) yield. ^{1}H NMR spectrum (400 MHz, DMSO-d_6): δ (ppm) 8.19 (d, *J* = 7.9 Hz, 1H), 4.74 (dt, *J* = 7.9, 5.5 Hz, 1H), 4.08 (dq, *J* = 11.3, 7.1 Hz, 4H), 3.64 (d, *J* = 14.3 Hz, 6H), 2.93–2.74 (m, 2H), 1.30–1.22 (m, 6H), 1.22–1.06 (m, 4H).

LC–MS data, m/z (*I*, %): exp. 366.108 $[MH]^+$, 100%; calc. 366.13 $[MH]^+$.

***N*-(α-diethoxyphosphorylcyclopropylcarbonyl)-methionine ethyl ester (9)**, 3.38 g (77%) yield. ^{1}H NMR spectrum (400 MHz, $CDCl_3$): δ (ppm) 8.05 (d, *J* = 7.5 Hz, 1H), 4.67 (td, *J* = 7.4, 4.9 Hz, 1H), 4.17 (m, 4H), 3.74 (s, 3H), 2.58–2.46 (m, 2H), 2.17 (tt, *J* = 14.2, 6.3 Hz, 1H), 2.08 (s, 3H), 2.06–1.95 (m, 1H), 1.51–1.42 (m, 2H), 1.35 (q, *J* = 7.3 Hz, 6H), 1.31–1.22 (m, 2H).

LC–MS data, m/z (*I*, %): exp. 354.336 $[MH]^+$, 100%; calc. 354.38 $[MH]^+$.

***N*-(α-diethoxyphosphorylcyclopropylcarbonyl)-γ-aminobutyric acid methyl ester (10),** 3.77 g (84%) yield. ^{1}H NMR spectrum (400 MHz, DMSO-d_6): δ (ppm) 7.71 (t, *J* = 5.5 Hz, 1H), 4.21–3.98 (m, 4H), 3.62 (s, 3H), 3.16 (q, *J* = 6.5 Hz, 2H), 2.42–2.29 (m, 2H), 1.70 (p, *J* = 7.1 Hz, 2H), 1.28 (t, *J* = 7.0 Hz, 6H), 1.24–1.01 (m, 4H).

Hydrolysis of the esters of N-(α-diethoxyphosphorylcyclo-propylcarbonyl)amino acids 12–15.

6.51 mmol of the ester of the *N*-(α-diethoxyphosphoryl-cyclopropylcarbonyl) amino acid was placed in a 50-mL flat-bottom flask; 7.9 mmol of KOH in the form of a 1 M solution were then added to the mixture under stirring. Stirring was carried out for another 24 h at room temperature. The potassium salt solution was then acidified with 20% HCl to reach a pH of about 3, and it was then stirred for 30 min at room temperature. At the end of the reaction, the solvent was removed by evaporation on a rotary evaporator. 50 mL of isopropyl alcohol were added to the resulting mixture, and the thus-formed precipitate was separated. The filtrate was concentrated on a rotary evaporator and cooled in a freezer until the desired product was observed to precipitate in crystalline form.

***N*-(α-diethoxyphosphorylcyclopropylcarbonyl)-glycine (12),** 1.27 g (70%) yield. Melting point: 75–77°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6): δ (ppm) 7.94 (t, *J* = 5.5 Hz, 1H), 4.19–4.01 (m, 4H), 3.83 (d, *J* = 5.5 Hz, 2H), 1.24 (t, *J* = 7.0 Hz, 6H), 1.22–1.00 (m, 4H).

LC–MS data, m/z (*I*, %): exp. 279.97 $[MH]^+$, 100%; calc. 280.24 $[MH]^+$.

***N*-(α-diethoxyphosphorylcyclopropylcarbonyl)-aspartic acid (15),** 1.18 g (54%) yield. ^{1}H NMR spectrum (400 MHz, DMSO-d_6): δ (ppm) 8.32 (s, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 4.58 (dt, *J* = 7.9, 5.0 Hz, 1H), 4.12–4.02 (m, 4H), 2.82–2.63 (m, 2H), 1.29–1.21 (m, 6H), 1.21–1.13 (m, 4H).

LC–MS data, m/z (*I*, %): exp. 338.111 $[MH]^+$, 100%; calc. 338.27 $[MH]^+$.

***N*-(α-diethoxyphosphorylcyclopropylcarbonyl)-methionine (13),** 1.56 g (68%) yield. ^{1}H NMR spectrum (400 MHz, $CDCl_3$): δ (ppm) 8.00 (d, *J* = 7.3 Hz, 1H), 4.65 (td, *J* = 7.2, 5.1 Hz, 1H), 4.17 (p, *J* = 5.7 Hz, 4H), 2.72–2.48 (m, 2H), 2.30–2.15 (m, 1H), 2.08 (s, 3H), 2.07–1.95 (m, 1H), 1.56–1.40 (m, 2H), 1.38–1.31 (m, 6H) 1.31–1.22 (m, 2H).

LC–MS data, m/z (*I*, %): exp. 354.336 $[MH]^+$, 100%; calc. 354.38 $[MH]^+$.

***N*-(α-diethoxyphosphorylcyclopropylcarbonyl)-γ-aminobutyric acid (14),** 1.28 g (64%) yield. ^{1}H NMR spectrum (400 MHz, $CDCl_3$): δ (ppm) 9.14 (s, 1H), 7.70 (t, *J* = 5.7 Hz, 1H), 4.14 (dt, *J* = 8.2, 7.0 Hz, 4H), 3.32 (q, *J* = 6.6 Hz, 2H), 2.36 (t, *J* = 7.3 Hz, 2H), 1.85 (h, *J* = 7.5 Hz, 2H), 1.54–1.39 (m, 2H), 1.32 (t, *J* = 7.1 Hz, 6H), 1.28–1.15 (m, 2H).

LC–MS data, m/z (*I*, %): exp. 308.059 $[MH]^+$, 100%; calc. 308.29 $[MH]^+$.

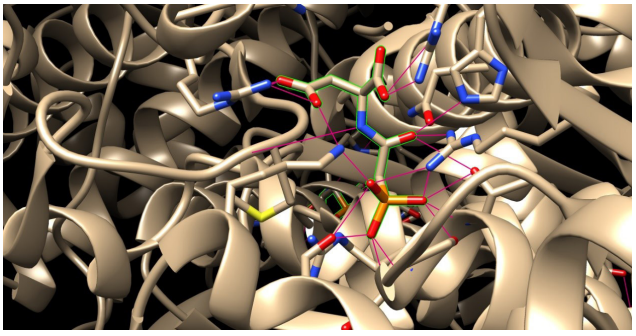
RESULTS AND DISCUSSION

In order to design a structure acceptable for binding to ATCase (Protein Data Bank code 5G1N[1]), we applied a molecular modeling method using the AutoDock Vina 1.1.2 program [10]. The optimization of geometric parameters was carried out using the molecular mechanics functionality in the Chimera 1.13.1 program[2]. Docking calculations made it possible to show that ATCase has a hydrophobic "pocket" where derivatives of phosphonacetic acid with cyclic substituents at the carbon atom can be incorporated with high probability (Fig. 3). Based on this method, it was also shown that the substitution of an amino acid residue in ATCase affects the value of the substrate–enzyme binding constant. Therefore, we conducted a series of experiments to obtain target *N*-(α-diethoxyphosphorylcyclopropylcarbonyl)-substituted amino acids with various amino acid residues.

The preparation of *N*-(α-diethoxyphosphorylcyclopropylcarbonyl)amino acid esters was carried out according to the scheme reported in Fig. 4. α-Diethoxyphosphorylcyclopropanecarboxylic acid **7** was obtained implementing a multi-stage synthesis whereby triethyl phosphite and ethyl chloroacetate were used as initial substrates; in fact, as a result of the Arbuzov reaction conducted under microwave irradiation conditions, the triethyl phosphonoacetic acid **5** was obtained [11, 12].

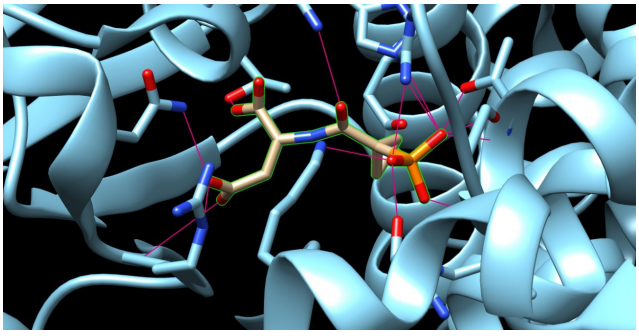
Taking into account the presence of an active methylene group in the structure of phosphonacetic acid and its derivatives, we obtained the complete ethyl ester of α-diethoxyphosphorylcyclopropanecarboxylic acid **6** as a result of an interphase alkylation reaction with 1,2-dibromoethane of phosphonoacetic acid triester, in the presence of dimethyl sulfoxide and potassium hydroxide. The complete ester of α-diethoxyphosphorylcyclopropanecarboxylic acid was then subjected to hydrolysis in an alkaline medium over the carboxyl component, which proceeded with the formation of α-diethoxyphosphorylcyclopropanecarboxylic acid **7** [13, 14].

The final stage of the synthetic procedure was the reaction of α-diethoxyphosphorylcyclopropanecarboxylic acid **7** with a number of amino acid esters: the esters of glycine and methionine, as well as the methyl esters of aspartic acid and γ-aminobutyric acid. The reaction was carried out through the preparation of the corresponding α-diethoxyphosphorylcyclopropanecarboxylic acid chloride, which was used further without preliminary isolation



a

Fig. 3a. An *N*-phosphonacetyl-L-aspartate molecule in the active site of aspartate transcarbomoylase (X-ray diffraction data; image obtained using the Chimera software).



b

Fig. 3b. An *N*-(α-dihydroxyphosphorylcyclopropylcarbonyl) aspartic acid molecule in the active site of aspartate transcarbomoylase (conformation modeled with Autodock; image obtained with the Chimera software).

[1] https://www.rcsb.org/structure/5G1N (Accessed July 01, 2020).
[2] UCSF Chimera 1.13.1. 2018. Available from https://www.cgl.ucsf.edu/chimera/ (Accessed July 20, 2020).

and purification. Carrying out the reaction under cooling to 0ºC in dry chloroform in the presence of triethylamine, which is used as a base and an acceptor of hydrogen chloride, enabled us to obtain esters of *N*-(α-diethoxyphosphorylcyclopropylcarbonyl)-substituted amino acids **8–11** in good yield.

The esters thus synthesized were converted into acids by alkaline hydrolysis of the carboxyl end of the compound (compounds **12–15**).

Note that in Fig. 5 are reported the compounds synthesized implementing the developed scheme, along with the corresponding yields for the last stage of the procedure.

Compounds **12–15** synthesized by us were tested for cytotoxicity toward cell lines of human breast cancer (MDA-MB-231, Fig. 6), skin cancer (A-375, Fig. 7), and glioblastoma (U-87 MG, Fig. 8), according to the method described in reference [15].

Cell viability was assessed via a test that is used to assess the metabolic activity of cells (the MTT test), based on colorimetric measurements of control and test solutions, which were preincubated in CO_2 environment with the addition of the MTT solution (3-(4,5-dimethylthiazole bromide)-2-yl)-2,5-diphenyltetrazolium). Nicotinamide adenine dinucleotide phosphate-H-dependent cellular oxidoreductase enzymes of living cells are able to reduce MTT to the corresponding formazan, which is characterized by a purple coloration. Subsequently, the optical density of the resulting solutions was estimated at 594 nm and 620 nm wavelengths. The results of these tests are reported in Figs. 6–8.

The absence of the expected biological activity of the test compounds may be due to the presence of ether groups at the phosphorus atom of phosphonate group [16]. Thus, one of the main directions of further research is the synthesis of *N*-(α-dihydroxyphosphorylcyclopropylcarbonyl)amino acids and verification of their biological activity.

CONCLUSIONS

The results of PALA studies and molecular docking of cyclopropanated analogs of phosphonacetic acid indicate that *N*-(α-dihydroxyphosphorylcyclopropylcarbonyl)-substituted amino acids have a high potential as anticancer agents. However, the absence of cytotoxicity of the synthesized compounds toward the cancer cell lines used in the present study did not match the expected results; the observed low

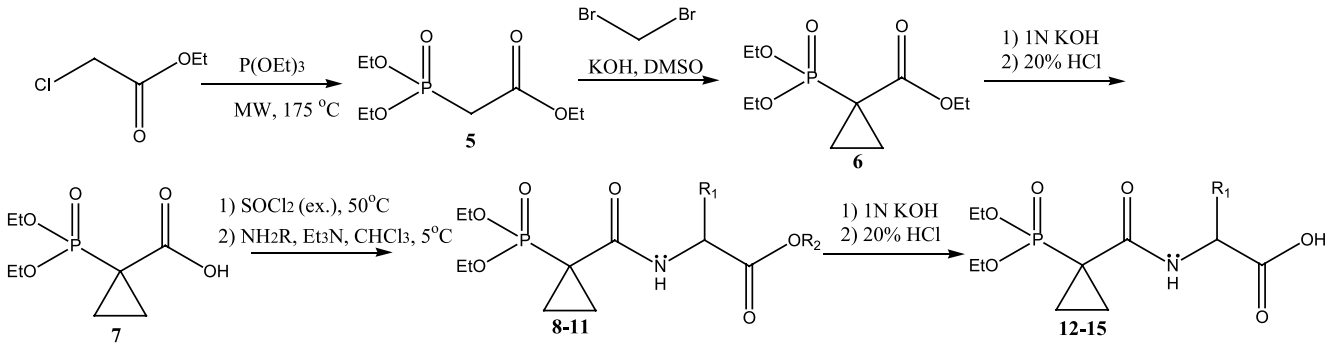


Fig. 4. General scheme for the synthesis of *N*-(α-diethoxyphosphorylcyclopropylcarbonyl)amino acids.

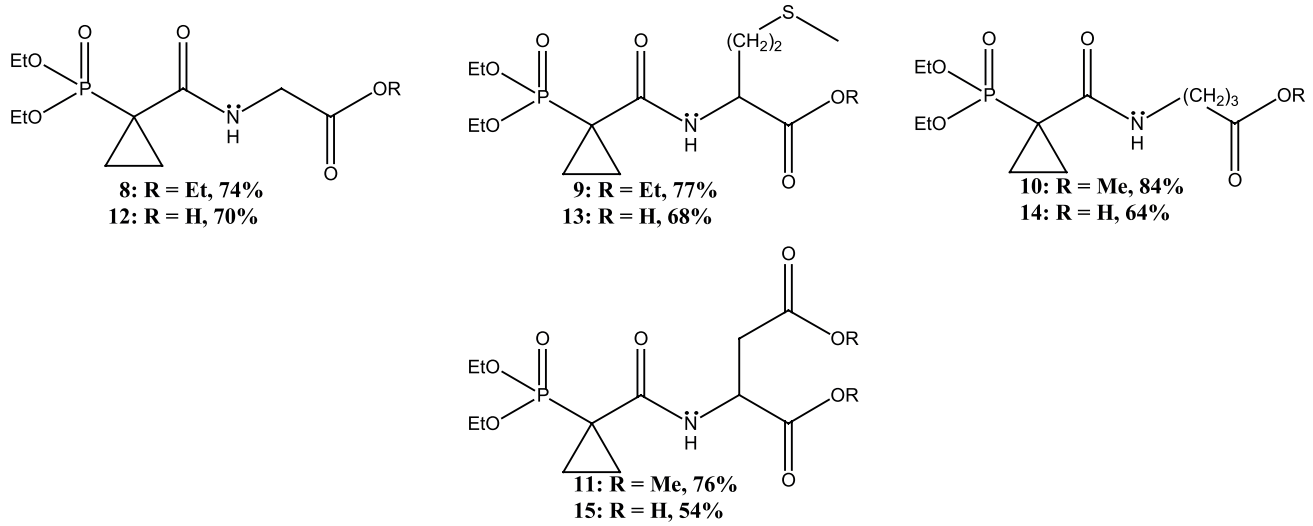


Fig. 5. A series of obtained *N*-(α-diethoxyphosphorylcyclopropylcarbonyl)amino acids, with their respective yields.

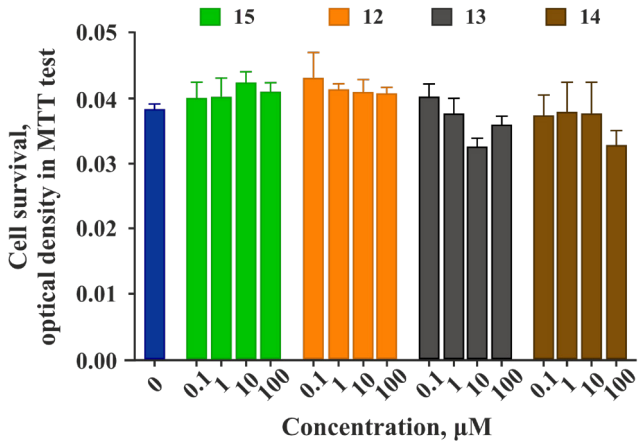


Fig. 6. Cell survival at various concentrations of the compounds under study as measured in cytotoxicity tests run against human breast cancer cell lines.

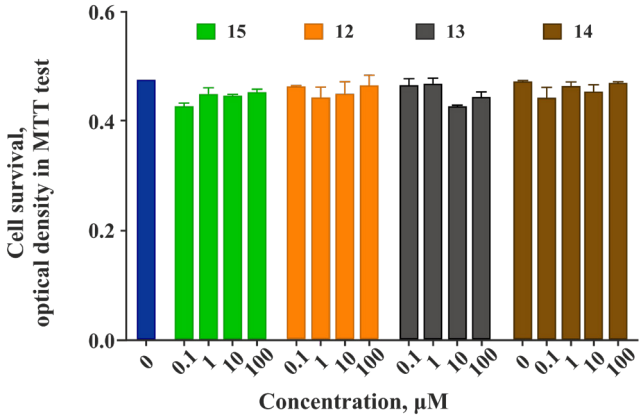


Fig. 7. Cell survival at various concentrations of the compounds under study as measured in cytotoxicity tests run against human skin cancer cell lines.

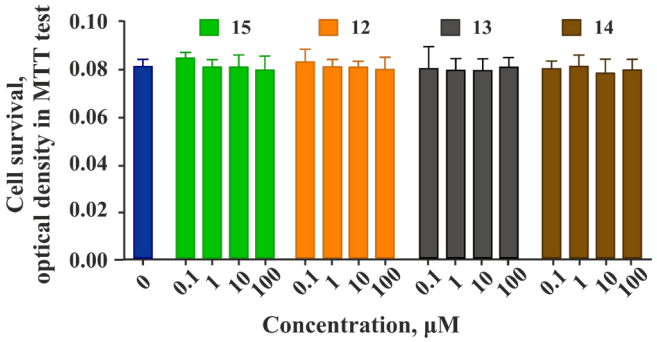


Fig. 8. Cell survival at various concentrations of the compounds under study measured in cytotoxicity tests run against human glioblastoma cell lines.

bioactivity may be due to the presence of ester groups in the phosphonate component of *N*-(α-diethoxyphosphoryl-cyclopropylcarbonyl)amino acids.

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Authors' contribution

I.S. Kuzmin – development of the research design, conducting the study, collecting and providing the material, writing the text of the article, editing the article;

D.Yu. Yuriev – conducting the study, collecting and providing the material, writing the text of the article, editing the article, formalization of the list of references;

G.A. Toporkov – conducting the study, collecting and providing the material;

A.V. Kalistratova – analysis and systematization of the material, writing the text of the article, design of the study;

L.V. Kovalenko – idea, development of the research design, analysis of the scientific work, critical revision with the introduction of valuable intellectual content.

The authors declare that they do not have any conflicts of interest related to financial or personal issues that could affect the work described in this article.

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